ACTIVATION ANALYSIS OF TRACE ELEMENTS IN SERUM

By

FREDERICK TURNER VARCOE, JR.

A THESIS PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1974

TO NILA

ACKNOWLEDGEMENTS

The author would like to thank his research director, Dr. Stuart Cram, for suggesting the research problem, for valuable advice, suggestions, and support, and for a wealth of practical experience. Thanks also go to the other members of the supervisory committee, Dr. Roger Bates, Dr. James Winefordner, Dr. Robert Hanrahan, and Dr. John James for their confidence and help. The project could not have been completed without them.

Thanks also go to the machine shop personnel, in particular to Mr. Arthur Grant, Mr. Chester Eastman, and Mr. Frank Ottinger for their expert fabrication of many pieces of equipment used in this work. The glassblowing skills of Mr. Truman Robbins, Jr., are also greatly appreciated.

The reactor operators and other personnel, in particular Mr. Henry Gogun at the University of Florida and Dr. Milton McLain, Jr., at Georgia Tech deserve heartfelt thanks for providing the irradiations necessary for these experiments.

The author also appreciates the contributions made by the other members of his research group. Dr. David Cottrell deserves special recognition for the design and assembly of the computer interface and software with which all the high-resolution gamma-ray spectra were recorded. Dr. Jack Leitner's expert help with the operation of the system and his encouragement and advice are also appreciated.

The author is deeply grateful to his parents for their support, encouragement, and sage advice throughout his education.

TABLE OF CONTENTS

Table of Contents Continued.....

Table of Contents Continued...

Table of Contents Continued...

Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

ACTIVATION ANALYSIS OF TRACE ELEMENTS IN SERUM

By

FREDERICK TURNER VARCOE, JR.

June, 1974

Chairman: Roger G. Bates
Major Department: Chemistry

This research deals with radiochromatographic separations in neutron activation analysis of trace elements in serum. Understanding the metabolic roles of trace elements as well as their toxicological effects in biological systems has been limited by the sensitivity and selectivity of existing analytical techniques. The lack of human serum concentration data for many trace elements prevents optimum clinical utilization of this extremely powerful diagnostic tool.

Neutron activation analysis has extremely low limits of detection for a large number of elements. However, interference from those elements present in human serum at relatively high levels must be eliminated before even a high-resolution solid state detector can be effective. The utilization of high sensitivity sodium iodide detectors requires still more separation.

Chromatographic separations of metal chelates were developed for both pre- and postirradiation separations. Quantitative elution from the gas chromatographic column for fluorine substituted beta-diketonate complexes of Cr, Cu, Mn, Fe, Be, Lu, and Gd was studied; recoveries between 52 and 92% were found. Extensive studies of decomposition and adsorption losses in the system are reported. Construction and evaluation of various sampling systems, transfer lines, and counting geometries are included. A completely reversible counting geometry for radioactive metal chelates using wide-range temperature and carrier gas control to trap and subsequently release eluate fractions is described. Also included is an apparatus for carrying out chelate separations from solutions whose large volumes preclude gas chromatographic injection.

The successful use of a chelating ion exchange resin in human serum neutron activation analysis is reported. Operating conditions which optimize sodium removal with respect to sample loss are described.

CHAPTER I

INTRODUCTION. TRACE ANALYSIS

Trace Elements in Serum

Most of the elements in the geochemical environment can be detected in animal and plant tissue if a sufficiently sensitive technique is available (1). Elements are present in bulk and trace concentrations. The trace elements, usually considered to be those present at concentrations below 100 ppm, can be divided into two groups: those which serve an essential biochemical function without which subnormal functioning or illness results, and those which are nonessential. Abnormally low concentrations of an essential element can cause a deficiency disease, while the lack of a nonessential element has no ill effects. Excess of either essential or nonessential elements can be toxic, however. As our knowledge of the biochemistry of trace metals grows, more elements may come to be considered essential. It is already known that many of those not now considered essential have very important roles in metabolism.

The concentrations of essential trace elements within the organism are maintained within very narrow limits, which is one reason why so much attention has been given to the relationships of these elements to normal and abnormal metabolic functions (2). Normal concentration ranges for nonessential

elements also exist, but are much broader than those of essential elements. The trace elements must be present in the diet. Their only sources are the earth's crust and the ocean. Life cannot exist without them (3).

Elements Identified

Those elements considered essential for healthy human biochemical functioning (1,4) are listed in Table 1, along with serum concentrations which have been found in normal human subjects. Also included are elements considered nonessential environmental contaminants, often having toxic effects. Reports dealing with elements whose serum concentrations are relatively high, or which for other reasons can be determined using less sophisticated analytical techniques, have existed for some years. Among these are Cu (5-48), Zn (19-59), Mn (14-27, 56, 59-62), Cr (13, 41-44, 57-71), I (47, 48, 58, 72-79) and Fe (11, 24-27, 40-42, 56, 58, 80-83). Recently new techniques have allowed identification of as many as 14 elements simultaneously in blood and serum samples (26, 40, 53), while V (84), Ba (85), Co (86), Sr (85, 87), U (88, 89), Ca (90-95), Mg (95, 96), Hg (97-99), As (99), Pb (100-102), Cd (101), Se (103-105), Au (106-108), Te (109), Li (110), W (111), Be (70, 112, 113), Br (114) and F (115), have been determined individually. Several review articles have been written on trace element analysis in biological systems (116, 117, 118).

Function

The importance of trace elements lies in their roles in

Table 1. Essential and Nonessential Elements in Human Biochemistry

Element	Serum Concentration µg/ml	Reference
Essential Bulk		
Ca	2.8	(116)
Cl	1,000	(116)
K	1,700	(116)
Mg	13	(116)
Na	3,250	(116)
P	0.50	(116)
S	5,400	(27)
Essential Trace		
Co	0.00018	(117)
Cr	0.02-0.05	(69)
Cu	1.16	(117)
F	0.028	(117)
Fe	1.14	(117)
I	0.0029-0.11	(117)
Mn	0.0083	(117)
Mo	0.4	(117)
Ni	0.0042	(117)
Se	1.1	(117)
Si	7.0	(27)
Sn	0.033	(117)
V	1.0	(117)
Zn	0.98	(117)

Table 1. continued...

Element	Serum Concentration µg/ml	Reference
Nonessential		
Ag	0.28	(116)
Al	5	(27)
As	0.19	(117)
Au	0.03	(116)
B	2	(27)
Ba	0.059-0.066	(116)
Be	<0.004	(117)
Br	830	(116)
Cd	<0.1	(117)
Cs	0.05	(27)
Hg	0.0027-4.0	(116)
La	18	(116)
Li	0.031	(117)
Pb	0.046	(117)
Rb	14	(27)
Sb	0.054	(117)
Sc	0.04	(116)
Sr	0.057	(117)
Th	0.4	(27)
U	0.048-0.072	(116)

biological functioning. They take part as active components of enzyme systems, in transport mechanisms, in tissue synthesis, and in bone formation (2).

The concentrations of trace elements in marine organisms are fixed by the concentrations of trace elements present in the surrounding water. In terrestrial animals, highly developed homeostatic processes are required to maintain trace element concentrations at fixed levels in the face of widely varying amounts in the environment. When, for one reason or another, an essential trace element's concentration lies outside the normal range, a serious metabolic upset must also be present.

Trace element concentration variations have been implicated in diseases ever since it became known that these elements were an integral part of metabolism. It has become obvious that knowledge of the function of trace elements in metabolism should help the diagnosis and treatment of disease (2).

Most diseases of any consequence are accompanied by changes in the concentrations of one or more trace elements in some tissue or body fluid. However, it must be kept in mind that only a few diseases have abnormal metal metabolism as a primary underlying cause, even though correcting the imbalance will sometimes alleviate the symptoms, e.g., oral Cr dosage will improve abnormal glucose tolerance, although abnormally low serum Cr probably does not cause diabetes (119). Even though many changes in the normal tissue concentration of a trace element are nonspecific indications of an abnormal

condition, the analysis of body fluids and tissues for trace elements is becoming an important clinical test in humans.

Blood is both the easiest to obtain for analysis and the most pervasive of the body's tissues. It travels everywhere in the body, providing an exchangeable reservoir of elements and compounds for all the other tissues. The component of blood which is most useful for trace element studies is the serum which remains after the blood cells and clotted fibrin have been removed by centrifugation.

Only a small fraction of the trace elements in serum are present as free ions. Most are in the form of complexes. They may be loosely bound to a ligand such as albumin, or tightly bound as in the iron-porphyrin compounds (2, 120). This provides a high degree of selectivity in the combinations available for metal complex formation (2, 120), and thus gives the body the means to select and accumulate the required metals from circulating body fluids. These complexes assist homeostatic mechanisms in maintaining constant trace element concentrations by forming or dissociating when necessary. Also, heavy metals can be maintained in the system at nontoxic levels while still providing the mineral reservoir needed for normal metabolism. The critical balance between metal ion and metal complex is easily upset by any of several factors which affect the stability constants of the complexes. Today scientists need to learn more about the biochemical phenomena that depend on these metals and more about the stability constants and specificity of the biochemical ligands. The serum concentrations of trace elements have the broadest

range of diagnostic applicability and therefore are of the most interest at this time from the standpoint of improving diesease diagnosis and etiological knowledge, and understanding trace metal metabolism in the human body.

Disease Relationships Established

Heart disease.-Heart disease is responsible for nearly half of the deaths in the United States each year. For this reason, it has received much study. Recently the possibility that trace elements play a part in cardiovascular diseases has become evident. Studies exploring this possibility show how trace element concentration information can play an important part in understanding and diagnosing a disease.

Injured heart tissue from patients who die from heart attacks (acute myocardial infarction) show abnormally low concentrations of Co, Cs, K, Mo, P, Rb and Zn and elevated concentrations of Br, Ca, Ce, La, Na, Sb and S (121). Serum levels in patients with myocardial infarction are also abnormal. Increases in serum Cu (11, 14, 15), Mn (14, 15), Ni, Mo and B (122) have been found, as well as a decrease in serum Zn (2, 123). Serum Cu determination has been suggested as a diagnostic test for patients with myocardial infarcation (11).

Trace element intake in drinking water has been linked with death from myocardial infarction. A highly significant negative correlation has been found between water hardness expressed as Ca ion concentration and the death rate in the local population due to degenerative cardiovascular disease in several countries, including the U.S. (124-126). Calcium

may only be a benign indicator of the presence or absence of another trace element which takes an active part in degenerative cardiovascular disease. For example, Ca is thought to inhibit the transfer of divalent metals across intestinal membranes (93). Perhaps this inhibition controls the presence of a trace element which takes an active part in the disease. Serum cholesterol has been linked to cardiovascular disease. The presence of trace quantities of V and Fe decreases cholesterol synthesis, while Cr and Mn have the opposite effect (127). Administration of $CaNa_2EDTA$ has been shown to reduce serum cholesterol and to increase urinary output of certain trace elements, especially Zn (123).

Cancer.-Trace metal studies have also been made in connection with cancer, another major cause of death in the U.S. Brune found no variation in the serum concentrations of 12 elements in leukemia patients (26), while other workers have found abnormal Fe (80) and elevated Zn (52) concentrations. High serum Cu concentrations are present with breast (12) and prostate (15) cancer, chronic and acute leukemias (2), and Hodgkin's disease (2). Hrgovcic as cited by McCall (2), gives an example of how determination of trace element levels can aid in evaluating the treatment of disease. In cases where malignancies are being treated, changes in the serum Cu concentration can appear long before there are any clinically apparent changes.

Studies attempting to correlate the death rate from cancer with various types of trace element environments in

different geographical areas have been made. Berg, in his study of carcinogenic trace metals in water supplies, states that although the literature contains some speculation, almost no convincing data exist with respect to these correlations (128). Pories, however, states that the large differences in death rate from cancer in different parts of the U.S. suggest that environmental factors may be the principal and preventable causes of cancer (129). He also states that elements can inhibit or foster tumor growth. Selenium and platinium interfere with tumor growth; iodine prevents thyroid neoplasia. Zinc metabolism may be the key for limiting tumor growth by manipulating necessary protein synthesis (129).

Other diseases.-The relationships between serum trace elements and other diseases have also received attention. Protein bound iodine determination in serum has been used for diagnosing abnormal thyroid activity for some time (72-79). Abnormal serum Cu and Zn concentrations have been linked with rheumatoid arthritis (31). In sera from arthritic patients Cu, Ba, Cs, Sn and Mo concentrations were elevated, Zn, Fe and Pb concentrations were depressed, and Al, Ni, Sr, Cr and Cd concentrations were normal (40). Hepatitis and liver cirrhosis are accompanied by elevated serum Zn and Mn (15, 49, 52). Serum Cr is depressed and Mn is elevated in individuals suffering from diabetes mellitus (15, 119). Oral Cr supplementation will improve such patient's glucose tolerance (15).

Zinc is required by more enzyme systems in the human body than any other metal (2). In general, decreases in

serum Zn are accompanied by increases in serum Mn, although this is not true in every case. Their antagonism has been noted in lymphoma, pneumonia, and other diseases (2).

Low Cu levels have been measured in patients with Wilson's disease (9), chronic and acute leukemias, aplastic anemia, cirrhosis, viral and microbial infections and nephritis (2). Elevated serum Zn accompanies psoriasis (30). Abnormal trace element concentrations have even been correlated with mental illness. Serum Zn and Cu concentrations have been measured in schizophrenics. Both are needed for histaminase enzymic action, variation of which has been associated with schizophrenia. The Cu concentration was found to be abnormally high, but returned to normal following effective therapy (33). Zinc and copper are antagonists in the human body. Oral Zn treatment has been used successfully with such patients, apparently forcing the Cu concentration down. Penicillamine, a chelating agent which removes trace metals from the body, has also been used for effective treatment (33).

Data from trace element studies using new analytical techniques can provide the basic information necessary for a better understanding of the roles of trace elements in diseased and healthy metabolic acitivity. These techniques can then be used to obtain the necessary information for individual diagnosis.

Serum Trace Element Analysis

Research on the metabolic roles and toxicological effects of trace elements in biological systems is highly dependent

on the development of new analytical techniques which are reproducible, rapid, sensitive, specific, and suitable for automation. The lack of such methods has thus far impaired development of the vast potential of serum analysis for diagnosis and treatment in the medical sciences.

Many instrumental methods for elemental analysis in serum have been reported, but the number well suited to trace analysis is small. The techniques reported have been limited in scope by several considerations. Matrix interference is severe. The Na concentration is 2-3 mg/ml, 10^4 times greater than even the higher trace element concentrations. With five or six other elements present at macro levels (Table 1), determining concentrations for the large number of trace elements present at ppm to sub-ppm levels requires advanced techniques, especially if highly desirable simultaneous multi-element analyses are to be achieved. The lower limits of detection presently available have undoubtedly limited the scope of previous determinations. The more popular trace element techniques, their advantages, shortcomings, and potential for serum analysis are discussed below.

Flame Spectroscopy

Flame spectroscopy is the most sensitive of the techniques requiring only moderately expensive equipment. More than 60 elements, including all the metals, can be determined this way. Interference is less likely than when using many other trace analytical techniques. Chemical interferences, those which prevent the atoms from reaching the free, dissoci-

ated, un-ionized state necessary for measurement in the flame, and spectral interferences due to bands emitted by the OH and CN radicals can present problems. Spectral interferences from other trace elements in the sample are seldom encountered. However, the total salt concentration in the liquid being analyzed should be kept below 1%, which can require sample dilution and adversely affect trace determinations (130). Tenfold dilution of serum samples has been found necessary by some workers to avoid clogging the burner (32). A solvent extraction step prior to analysis can eliminate the need for dilution, although the risk of contamination is greatly increased. Whatever separations are required prior to determination are usually simple (131).

Probably the main disadvantage flame spectroscopic techniques have is that except in the case of atomic emission, only one or two elements can be determined at a time. Research is currently under way to remedy this, but until these systems become fully operational either another technique must be used or one must make a separate series of measurements for each element.

Atomic absorption (AA), atomic emission (AE), and atomic fluorescence (AF) are complementary spectroscopic techniques. When the limit of detection must be optimized, AA and AF are generally preferred for elements with resonance lines below 300 nm, since there is usually insufficient energy available in a properly adjusted flame to excite these higher energy transitions. For elements with resonance lines above 400 nm,

there is adequate flame energy available and an external radiation source is not required. Elements whose resonance lines lie between 300 and 400 nm can usually be determined equally well by any of the three techniques (130).

Atomic absorption.-Those elements for which AA has a lower limit of detection than AE by at least a factor of 5 are listed in Table 2. The classification is based on comparison, as flame techniques, without regard for the fact that in some cases where sample size is limited, and in other cases where the nonflame technique is unusually sensitive, a lower limit of detection may be obtained with nonflame AA.

Table 3 lists those elements for which AA and AE have limits of detection which are comparable. Together these lists contain 51 elements. When Table 1 is compared with Tables 2 and 3, it can be seen that of those essential trace elements which can be determined spectroscopically all have AA detection limits equal to or lower than the corresponding AE limit. With this in mind, it is not surprising that AA has been the most popular spectroscopic technique for trace element analysis of serum. Of course, classification as essential requires an adequate method for detecting the presence of the element in question, and as other techniques are applied to trace element analysis of biological samples the essentiality of other elements may be discovered at levels too low for analysis by AA.

A few representative determinations of trace elements in serum by AA include Ca (90, 94); Fe, Cu and Zn (32, 34,

50); Au (108); Li (110); and Pb (77). Two of these references, Au (108) and Li (110), describe metal determinations in sera of patients currently undergoing therapy with these metals and therefore involve abnormally high concentrations. Techniques which require only microliter samples are being used more frequently for serum analysis. The Delves cup technique has been applied to serum chelate extracts of Pb and Cd (102), and the carbon rod atomizer has been used to determine Mg, Fe, Cu, Pb and Zn concentrations in serum (35). These techniques are especially valuable where multielement determinations are required, since until simultaneous multielement spectroscopic determinations are improved, a separate portion of the sample will have to be used for each element determined.

Atomic emission.-Those elements for which AE has a lower limit of detection than AA by at least a factor of 5 are listed in Table 4. None of the essential trace elements appears on this list, although several of the bulk essential elements do. Tables 3 and 4 combined list 43 elements, including five essential trace elements, for which the AE limit of detection is at least as low as the AA limit of detection. AE, then, can be a valuable flame spectroscopic tool in serum trace metal studies, both because it is best for several elements presently known to take an active part in metabolism, and because it can be used for studying elements for which AA is not adequate. Besides this, it may also provide a basic technique for multielement analysis. For example, eight elements have been determined in blood using plasma torch excitation of an emission spectrum (138).

Table 2. Elements for Which AA Has a Detection Limit Lower than AE by at Least a Factor of 5

Element	Lower Limit of Detection Flame AA, µg/ml		Nonflame AA, ng	
As	0.1	(131)	0.2	(132)
Au	0.01	(133)	0.07	(134)
Be	0.002	(131)	0.003	(134)
Bi	0.5	(131)	0.02	(134)
Cd	0.0006	(133)	0.00006	(134)
Co	0.005	(131)	0.007	(134)
Fe	0.005	(131)	0.02	(134)
Ge	0.1	(133)	-	-
Hf	15	(135)	-	-
Hg	0.2	(133)	0.4	(134)
Ir	4	(135)	-	-
Mg	0.0003	(131)	0.003	(134)
Ni	0.005	(131)	0.02	(134)
Pb	0.03	(131)	0.02	(132)
Pt	0.5	(135)	0.2	(134)
Rh	0.03	(131)	0.06	(134)
Sb	0.07	(133)	0.05	(134)
Se	0.1	(131)	0.2	(134)
Si	0.1	(131)	0.003	(134)
Sn	0.02	(131)	0.001	(134)
Te	0.1	(131)	0.008	(134)
Ti	0.1	(135)	0.5	(134)
Zn	0.002	(131)	0.001	(134)
Zr	5	(135)	-	-

Table 3. Elements for which AA and AE have Comparable Detection limits

Element	Lower Limit of Detection, µg/ml	Technique	
Ag	0.005	AA	(131)
Ba	0.03	AE	(135)
Cr	0.005	AA-AE	(131,136)
Cu	0.003	AA	(133)
Dy	0.1	AE	(135)
Er	0.2	AA	(135)
Gd	2	AE	(135)
Ho	0.1	AE	(135)
K	0.003	AE	(135)
Mn	0.002	AA	(131)
Mo	0.03	AA	(131)
Nd	1	AE	(135)
Pd	0.02	AA	(133)
Pr	2	AE	(135)
Rb	0.002	AE	(135)
Re	1	AE	(135)
Ru	0.3	AA-AE	(135)
Sc	0.07	AE	(135)
Ta	6	AA	(135)
Tb	1	AE	(135)
Tl	0.02	AA-AE	(105, 108)
Tm	0.1	AA	(135)
U	10	AE	(135)
V	0.01	AE	(136)
W	3	AA	(135)
Y	0.3	AA-AE	(135)
Yb	0.04	AA	(135)

Table 4. Elements for Which AE has a Detection Limit Lower than AA by at Least a Factor of 5

Element	AE Detection Limit, ug/ml	
Al	0.005	(137)
Ca	0.0001	(136)
Ce	10	(135)
Cs	0.008	(135)
Eu	0.003	(135)
Ga	0.01	(136)
In	0.002	(135)
La	1	(135)
Li	0.000003	(135)
Lu	0.2	(135)
Na	0.0001	(135)
Nb	1	(135)
Os	10	(135)
Sm	0.6	(135)
Sr	0.0002	(136)
Th	150	(135)

Atomic fluorescence.-AF is the newest of the atomic spectroscopic methods. According to Winefordner et al. (139), for a population of atoms in an atom reservoir with low background the sensitivity of AF measurements is inherently greater than that of AA because of benefits gained from signal amplification and increased source intensity. This greater sensitivity has been applied to serum analysis by a few workers, such as Kolihova and Sychra (7), who determined Cu. As source stability and intensity problems are overcome, AF will become more popular for trace analysis.

Arc-Spark Emission Spectroscopy

Emission spectroscopy is capable of determining up to 70 elements with absolute lower limits of detection down to 10 ng (140). For serum, sample preparation consists only of dilution if the Stallwood Jet or similar apparatus is used, or dry ashing if a conventional graphite cup electrode is used. No separation steps are required. Matrix interference is not a problem, especially if the cyanogen bands have been eliminated by using an arc stand with an inert atmosphere. Besides these advantages, simultaneous multielement determination is possible, an important consideration when only a small amount of sample is available for a survey-type determination. Drawbacks include the difficulty of obtaining quantitative data of even limited accuracy. Photographic emulsions require troublesome calibration, and tedious and time consuming development. Direct readers eliminate this, but allow observation of only a limited number of wavelengths. These

must be chosen before analysis, thereby limiting the technique even further. Direct readers also add significantly to the already large expense associated with emission spectroscopy.

Monacelli et al. (46), and Paixo and Yoe (45) determined Cr, Cu, Mg, Ni and Zn in plasma using emission spectroscopy. Nedermieier and Griggs have used a direct reading emission spectrometer to determine the concentrations of 14 trace elements in serum (40).

Colorimetry and Fluorimetry

These two techniques can be very simple, sensitive, and require only inexpensive apparatus under certain ideal conditions. Unfortunately, since hardly any of the organic reagents used with these techniques are specific for only one element, interference is a major problem. Complex separation schemes can be necessary, especially for complex samples such as serum, thereby removing any benefits from the simplicity of the technique.

Colorimetry.-Trace quantities of elements in serum determined colorimetrically include I (78), Br (112), Fe (81, 82), and Cu (6). In their review of trace element analysis in clinical chemistry, Schroeder and Nason (117) include some lower limits of detection for several elements using colorimetry. These are shown in Table 5. Where the limit of detection is specified in µg rather than µg/g, the figure indicates the smallest amount detectable by difference in two solutions, both with known amounts added (117).

Table 5. Colorimetric Lower Limits of Detection

Element	Limit, (µg/g)
Essential	
Cr	0.006
Mn	0.01 µg
Mo	1.0 µg
Ni	0.05
Sn	5.0 µg
V	0.5 µg
Nonessential	
As	0.5 µg
B	0.5
Ge	0.1
Nb	0.04
Pb	0.05 µg
Ti	0.25 µg
Zr	0.8

Fluorimetry.-Since the lower limit of detection of any spectroscopic technique is more a function of the signal-to-noise ratio than signal strength alone, the limits of detection for inorganic fluorimetry are usually much lower than those for inorganic absorption spectrophotometry (141). The limits of detection in interference-free media for several elements as noted by Winefordner et al. are listed in Table 6. These limits show that fluorimetry can be comparable to other sensitive methods of trace analysis if interference is not a problem. Interference in complex samples such as serum is greater in fluorimetry than in AE, AA or AF, however.

X-Ray Techniques

X-Ray fluorescence.-Elements with atomic numbers above 15 can be effectively determined at ppm levels by X-ray fluorescence. Recent reductions in the size of X-ray sources, lower detector background, and improved resolution and counting rate capabilities of detectors and associated electronics have greatly increased the capabilities of the technique (142).

Semiconductor detectors capable of surveying an entire spectrum of trace elements present at levels below 1 ppm have been used in serum analysis (143, 144). Analyses of petroleum (145) and air filter particulates (146, 147) have demonstrated absolute detection limits down to 10 ng/cm^2 for small particles. These levels cannot be reached with serum without first concentrating the dissolved solids to a greater degree than is provided by drying alone. Ion exchange membranes (145) and chelating ion exchange resins (148) provide

Table 6. Fluorimetric Lower Limits of Detection (141)

Element	Limit, µg/ml	Element	Limit, µg/ml
Ag	0.004	Li	0.2
Al	0.0008	Lu	100
As	7	Mg	0.00001
Au	0.5	Mn	0.002
B	0.0005	Mo	0.1
Be	0.00004	Nb	0.1
Bi	0.5	Nd	5
Ca	0.01	Ni	0.00006
Cd	0.02	Os	0.05
Ce	0.05	P	0.0000006
Cl	0.05	Pb	5
Co	0.0001	Pr	0.5
Cu	0.0002	Ru	1
Dy	0.01	Sb	0.05
Er	10	Sc	0.01
Eu	0.005	Se	0.005
F	0.001	Si	0.08
Fe	0.0008	Sm	0.5
Ga	0.001	Sn	0.1
Gd	10	Tb	0.1
Ge	0.004	Te	0.2
Hf	0.1	Th	0.02
Hg	0.002	Tl	0.02
Ho	100	Tm	10
I	0.6	U	0.01
In	0.04	V	2
Ir	2.0	W	0.04
		Y	0.02
		Zn	0.002
		Zr	0.02

both preconcentration and thin geometry. Cobalt determination at 0.001 ppm (145) and Cr(VI) and/or Cr(III) determination at microgram levels (147) have been carried out in this manner.

Quantitative analysis in a matrix as complex as serum requires complicated mathematical manipulation of the data to compensate for the X-ray enhancement and absorption effects of other elements in the sample. These interelement effects are compensated for by solving sets of simultaneous equations containing measured constants for each element present (149). Losses of sensitivity also occur if higher spectrometer resolution is required for analysis of complex mixtures. This becomes even more significant if the high resolution of less sensitive dispersive systems is required.

Electron probe and laser probe X-ray microanalysis.-This technique has been applied successfully to the analysis of small regions in hetreogeneous samples. Its relative lower limits of detection are not outstanding, but absolute limits as low as 10^{-2} pg have been reported (145). Since the mass of a serum sample after dry ashing and sodium removal is very low, this technique may be successfully applied.

Mass Spectrometry

Spark source mass spectrometry.-For trace element qualitative and semiquantitative analysis, this technique is unsurpassed in cases where both low limits of detection and a wide range of elements analyzed are required. Interferences are not severe, thus allowing lower limits of detection to range

from 0.1 to 0.001 ppm for most elements in any matrix (150). The small sample size requirement makes this technique especially useful for serum analysis, where the mass of the ash to be analyzed is quite low.

The most serious drawback of this technique is its poor quantitative accuracy. The precision is limited to about 25% (151). The discharge is unstable, thus preventing spectrum scanning and requiring photographic detection. Ion yield is influenced by sample preparation, thus making comparisons between natural samples and synthetic standards unreliable. Lack of good quantitative data combined with the expense associated with purchasing and maintaining the instrument are reasons why little trace element determination in serum has been done.

One of the few publications using this technique is by Wolstenholme (27), who determined 25 elements in plasma. The reported concentrations deviate from accepted values by factors as large as 100, thus illustrating the difficulty of accurate quantitative analysis.

Isotope dilution mass spectrometry.-Much of the uncertainty in qualtitative mass spectrometry mentioned above can be eliminated by using isotope dilution. No reference standards are required, and the technique is sensitive and specific. Isotope dilution allows precise quantitative determination because only isotope ratios from lines recorded under identical matrix, exposure, development, and emulsion conditions are measured. All the uncertainty due to lack of reproducibility is eliminated.

Early application of the isotope dilution technique with electron bombardment sources was limited by element volatility. The technique has been extended to less volatile metals by Frew et al. (152), who determined Cr as a β-diketonate.

Isotope dilution can also be used with an rf spark source (153). This involves additional sample preparation, as does a chelation step. The care required in the tedious steps, as well as equipment expense keep this technique from being popular for serum analysis, although some serum analyses have been reported (154).

Electrochemical Methods

With the exception of potentiometry, electrochemical methods have not been widely used for trace element determinations in biological media. For example, in a recent Analytical Chemistry review article which dealt with biological trace analysis only one article of the 49 reviewed dealt with a nonpotentiometric electrochemical determination (155). One reason for this is the susceptibility of electrochemical techniques to interference. Anodic stripping voltammetry, for example, can be applied to extremely low concentrations and is still sufficiently selective to allow separation from species present in a thousandfold or greater excess (156). Even so, multielement serum trace analysis cannot be carried out for elements with similar unmasked electrodeposition potentials. Because only a 1.5-V anodic dissolution "window" exists between the cathodic deposition potentials and the

anodic water-salt breakdown potential limit, one cannot obtain separate and distinct dissolution peaks for the large number of different ions present in serum (156).

Potentiometry with ion selective electrodes has been applied to serum analysis. Serum calcium, a bulk constituent, has been determined (91, 92) as has serum fluorine (115). As sufficiently sensitive ion selective electrodes become available for more elements, more such determinations can be made.

Czaban and Rechnitz (157) have constructed ion-selective electrodes with tip sizes in the 100-150 μm range. Microelectrodes such as these are capable of making in situ measurements in biological systems. Even though concentration measurements as low as 10^{-5} M can be made (157), in situ serum trace element determination may not be possible due to the strong metal binding properties of the serum proteins. The protein complex formation constants must be known and the extremely low free ion concentration must be measured before a meaningful concentration figure for a given element can be obtained.

Coulometry has received limited use in biological trace element analyses. Selenium in urine (158) as well as chromium in serum (71) has been determined. As little as 0.2 μg of chromium was detected.

Gas Chromatography

Gas-liquid chromatography is one of the most widely used methods of qualitiative and quantitative organic analysis, as well as an extremely powerful tool for separation prior to

analysis by other techniques. The chemical similarity of the compounds being chromatographed poses no problem, since separation depends only on very small differences in the volatility and solubility of the compounds in the liquid partitioning phase. Instrumentation can be inexpensive, yet provide analyses which would be difficult, if not impossible using another method. Extraordinary sensitivity, selectivity, ease of operation, and speed of analysis are some of the characteristics of gas chromatographic separations. The extremely high resolution available with long columns is unsurpassed by nonchromatographic separation methods. Optical isomers, geometrical isomers, even isotopes have been separated by gas chromatography.

For several years after its initial development this powerful analytical tool was used exclusively for organic compounds. This was due to the lack of suitable volatile, thermally stable metal compounds. In 1959, Wachi (159) and Freiser (160) reported gas chromatographic metal chloride separations, and Duswalt (161) reported separation of metal acetylacetonates. From this point metal analysis by gas chromatography has grown to be the well established technique it is today.

Metal chlorides.-Some metals can be separated by gas chromatography in the pure state. Sokolov et al. (162) have separated cadmium and zinc vapor at 850 °C. Such separations are difficult, requiring severe conditions. The use of volatile metal chlorides allows separation at much lower tempera-

tures. The chlorides of Ni, Sb, Sn, Ta and Ti are sufficiently volatile for elution. Separations of Sn and Ti, Sb and Ti, and the gas chromatographic behavior of ten other metal chlorides have been reported (163). Sie, Bleumer and Rijnders (164-166) have studied the gas chromatography of 12 elements, all of which were found to elute satisfactorily. The boiling points of the chlorides studied are listed in Table 7. All but two boil below 200 °C and are eluted at 125 °C after reasonable periods of time (164). Metal halides can require moderately high temperatures for elution. Their reactivity, especially their hygroscopicity, presents problems in sample handling and choosing adequate column packing materials.

Organometallic compounds.-Most classes of volatile metal compounds are organometallic in nature. Among these are metal alkoxides, carbonyls, alkyls, hydrides and π-bonded metal complexes such as cyclopentadienyls (167). Metal alkoxides, such as Al, Ge, Si and Ti isopropoxides, as well as Hf, Ti and Zr tertiary-amyloxides, have been eluted with little decomposition at moderate column temperatures (168). Most of the rest have proved unsatisfactory for various reasons. Metal carbonyls, hydrides and alkyls rarely form in quantitative or easily reproducible yields. In addition, solvolysis during formation and in the gas chromatographic liquid phase is often encountered with all of the classes mentioned.

Metal chelates.-Of all the various classes of metal compounds, metal chelates are the most useful for metal analysis by gas chromatography. This is due to the ease with

Table 7. Boiling Points of Various Chlorides

Element	Substance	Boiling point, °C
C	CCl_4	76.7
Si	$SiCl_4$	57.0
P	PCl_3	76
	$POCl_3$	105.3
S	SCl_2	59
	S_2Cl_2	138
	$SOCl_2$	75.7
	SO_2Cl_2	69.2
Ti	$TiCl_4$	135.8
V	VCl_4	152
	$VOCl_3$	127
Cr	CrO_2Cl_2	117
Ga	Ga_2Cl_6	200
Ge	$GeCl_4$	83.1
As	$AsCl_3$	130
Sn	$SnCl_4$	113
Sb	$SbCl_3$	221
	$SbCl_5$	

which they form in quantitative yields and their general lack of hydrolysis.

There are several ligands whose chelates have been studied using gas chromatography. The 8-hydroxyquinolate complexes of Al(III), Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Ga(III), In(III), Mg(II), Mn(II), Ni(II), Pb(II) and Zn(II) will sublime at reduced pressure at temperatures between 250 and 500 °C leaving no significant residue (169). However, their chromatography is complicated by a lack of suitable high temperature packings.

Benzoyltrifluoroacetone [H(tba)] and thenoyl-trifluoroacetone [H(tta)] have been used as ligands with Al, Cr, Cu, Fe and Ga in successful gas chromatographic separations (170). Pivaloyltrifluoroacetone [H(pta)] chelates of Y, Sc, and nine rare earth elements have also been successfully eluted (171).

Metal chelates of the β-diketones are the best choice for gas chromatography of metals thus far studied. They form with quantitative yields, several metals in a mixture being chelated simultaneously and reproducibly with ease. In most cases solvolysis and hydrolysis are not significant. Besides this, fluorinated derivatives yield chelates whose vapor pressures are low enough to permit gas chromatography without appreciable decomposition, while taking advantage of the extreme sensitivity of the electron capture detector for electronegative elements. Most fluorinated β-diketonates will rapidly sublime at reduced pressures at temperatures from ambient up to 100 °C.

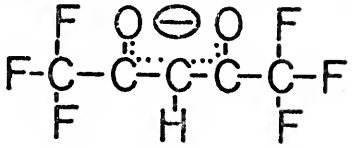
Rare earth chelates of several β-diketone ligands have been successfully eluted. These include 2,2,6,6-tetramethyl-3,5-heptanedione [H(thd)] (172), 1,1,1-trifluoro-5,5-dimethyl-2,4-hexanedione [H(tapm)] (173) and 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione [H(fod)] (174). All three formed anhydrous, stable chelates suitable for gas chromatography, but the fod chelates appear to be the most useful.

The ability of several β-diketones to form efficiently extracted, completely eluted chelates of Zn and Co has been studied. The ligands compared were H(fod), 1,1,1-trifluoro-2,4-pentanedione commonly called trifluoroacetylacetone [H(tfa)], 1,1,5,5,5-hexafluoro-2,4-pentanedione commonly called hexa-1 fluoroacetylacetone [H(hfa)], 1,1,1,2,2,6,6,6-octafluoro-3,5-hexanedione [H(ofhd)], and 1,1,1,2,2,3,3,7,7,7-decafluoro-4,6-heptanedione [H(dfhd)]. The order of extraction efficiency from aqueous solution into benzene was H(fod)>H(dfhd)>H(ofhd)> H(tfa)>H(hfa). Even though H(fod) extracts more efficiently than the rest, the fact that H(dfhd) forms chelates much more rapidly (10 min versus 4 hr for the Zn complex) makes it more desirable for some applications (175).

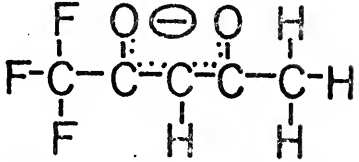
Three of the most successful of the fluorinated β-diketone ligands are H(tfa), H(hfa), and H(fod). Their anions are shown in Figure 1. Their chemistry is discussed below.

H(tfa).-Trifluoroacetylacetone is easy to work with, forming many stable chelates. H(tfa) boils at 107 °C and is soluble in most organic solvents. It is slightly soluble in water. The ease with which tfa chelates are formed is typical

hfa 1,1,1,5,5,5-hexafluoro-2,4-pentanedionate



tfa 1,1,1-trifluoro-2,4-pentanedionate



fod 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionate

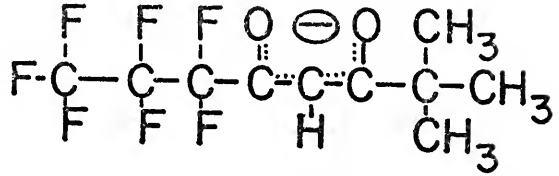


Figure 1. The Anions of the Fluoro-acetylacetone Ligands

of the β-diketones. Solvent extraction is applicable to most samples and yields a chelate solution which can often be injected into the gas chromatograph without further treatment.

If sample dissolution is required, either nitric or perchloric acid should be used. This avoids adding anions which can compete with the chelating agent for the metal ion.

Next the pH of the solution must be adjusted. For some extractions there is a wide range of pH values within which the optimum extraction efficiency is obtained. Genty et al. (176) found 100% extraction of $Al(tfa)_3$ between pH 5 and 9. Scribner et al. (177) found essentially 100% extraction of $Cu(tfa)_2$ between pH 4 and 8, and of $Fe(tfa)_3$ between pH 4.5 and 6.1. In a later paper Scribner et al. (178) report 99.9% extraction for $Be(tfa)_2$ between pH 5 and 7. A sharp rise in extraction efficiency around pH 3 and a high plateau continuing to at least pH 7 is typical of tfa chelates of trivalent metal ions. The divalent ions of Mg, Mn, Ni and Zn are all poorly extracted as tfa's in the pH 3 to 7 region due to hydrate formation.

In some cases the pH adjustment is critical. This is particularly true where variations in pH are to be used to carry out the separation of groups of metals prior to gas chromatographic analysis.

Some metals form insoluble hydroxides which can interfere with chelate formation. Cu can be extracted efficiently from solutions cloudy with $Cu(OH)_2$, but Fe does not extract

cleanly when initially present as an hydroxide (177). One way to circumvent this problem when dealing with trace concentrations is to keep the pH so low that the solubility product constant for the hydroxide is not reached. If this cannot be done within the pH range of efficient extraction, weak auxiliary complexing agents such as acetate ion can be used. Some neutralization of the acidic H(tfa) is often required. Acetate salts can thus bring the pH up to a suitable level while preventing hydroxide formation. One drawback is that if a large variety of ions is being extracted, some may be sequestered by the acetate ion and not extracted at all. Sequestration has been shown to occur with ammonia at pH 9 and higher, as well as with EDTA at pH 5 (177).

Since there is competition for the basic sites on the ligand anion, complex formation is favored at higher pH. The pH also affects ligand solubility. The larger the concentration of ligand in the aqueous phase, the farther the chelate formation reaction will proceed. This consideration is not critical with H(tfa), since its distribution between the organic phase and the aqueous phase can favor chelation at pH values as low as -0.5, with $D_{o/w} = 2.9$ (177).

The organic solvent chosen for the extraction should have a high chelate solubility to avoid chelate precipitation in the aqueous phase. If an electron capture detector is to be used for gas chromatographic detection, a hydrocarbon such as benzene for which detector response is slight can be used to advantage. Chelation and extraction generally

occur immediately after the ligand-organic solvent mixture is introduced. Excess ligand and an additional extraction step are usually helpful in ensuring complete extraction. The solid chelate can be obtained by evaporating the solvent. Alternatively, an aliquot of the solution can be injected directly into the gas chromatograph.

While solvent extraction is the oldest and best established technique for forming metal chelates for analysis by gas chromatography, faster and less complex methods are being developed. Ross and Sievers (179) report a direct chelation procedure used for the analysis of trace chromium in steel. A metal particle to be analyzed is placed in a glass tube with a small amount of H(tfa) and a drop of HNO_3 as a catalyst. Next the sealed tube is heated for 30 min with a microwave generator. The use of microwave energy stimulates reaction at the metal-solution interface without overheating the major part of the unreacted ligand and risking decomposition. The solution is then dissolved in benzene and the excess ligand, along with $Fe(tfa)_3$, is removed.

Sievers et al. have studied the suitability of various metals for chelation and gas chromatographic analysis (180). Their results are shown in Table 8. Class I is made up of chelates chromatographed with no evidence of decomposition in the injection port or column. Sharp, well defined peaks were obtained. Class II compounds, although eluting with sharp peaks, left residue in the injection port, indicating partial decomposition.

Table 8. Metals Suitable for Gas Chromatography

Class I	Class II
Al	Hf
Be	Mn
Cr	Zn
Cu	Zr
Fe	
Ga	
In	
Rh	
Sc	

Metal analysis by gas chromatography has fewer interference problems than most other techniques because the analysis process involves separation. Interference in the analysis of various tfa complexes has been studied. Ross and Sievers (181) found no interference with ppm Be analyses from a mixture of seven metal cations, most of which form stable chelates with H(tfa). A mixture of five anions also failed to interfere. It has also been shown that quantitative extraction of some metals, including Be, can occur in the presence of EDTA (178). If interference at high concentrations should pose a problem, the interferents could probably be effectively masked by EDTA.

Genty et al. (176) found some metals interfered to a small extent in the analysis of microgram quantities of Al in uranium. The interferents were present at concentrations 100 times greater than the Al, yet only errors below 25% were encountered.

Savory et al. (67) tested various ions for interference in the analysis of Cr as $Cr(tfa)_3$. The ions tested were As, Pb, Hg, Li, Mn and Sr at 50 µg/100 ml; K at 40 mg/100 ml; Ba, Cu, Ni, and Zn at 100 µg/100 ml; Ca at 10 mg/100 ml; and $(H_2PO_4)^-$ at 5 mg/100 ml. The presence of these ions had no effect on the Cr determination.

The usefulness of H(tfa) has been shown in many analyses. A few representative analyses include Al, Fe and Cu determined simultaneously in alloys (177, 182); Fe and Al (183) and Al, Ga and In (184) determined using the solvent extraction

procedure; microgram quantities of Al, Ga, In and Be determined with an overall relative mean error of 2% (185); and Be, Al, and Cr (186), as well as Al in uranium, determined at levels down to 10^{-11} g (176).

H(hfa).-Hexafluoroacetylacetone boils between 63 and 70 °C and is soluble in most organic solvents. Since H(hfa) is more highly fluorinated than H(tfa), its chelates have higher vapor pressures. This allows the hfa chelates to elute at lower column temperatures, thereby allowing more thermal instability. $Cr(hfa)_3$, for example, can be eluted rapidly at column temperatures as low as 30 °C. This temperature would have to be more than 100 °C higher to elute the nonfluorinated chelate under similar conditions (187).

Unlike H(tfa), H(hfa) reacts with water to form a tetrahydroxy compound, 1,1,1,5,5,5-hexafluoro-2,2,4,4-tetrahydroxypentane. This makes synthesis of hfa metal chelates from aqueous solutions of the metals much more complicated than the tfa synthesis and extraction described above. Although some aqueous hfa syntheses have been reported, they are probably best avoided. One alternative is conversion to the metal chloride followed by direct reaction with the ligand to yield the chelate and HCl. This is more time consuming and awkward than the extraction procedure.

Hill and Gesser (186) successfully performed quantitative analysis of Be, Al and Cr as hfa chelates. Cr has been determined as the tfa chelate in the 10^{-3} - 10^{-8} g range (188),

while Cr and Al have been determined at the 10^{-11} g level (189). Moshier and Sievers list Al, Be, Cr, Rh and Ti as having been successfully eluted as hfa complexes without any trace of decomposition (190). The hfa complexes of Ni, Ta and Ti have been successfully chromatographed, but only with great care to prevent reaction with moisture in the air. It has been shown that $Cu(hfa)_2$ will deposit elemental Cu on the walls of a glass tube through which its vapor is passed at 275 °C. In a hydrogen atmosphere this deposition takes place at 250 °C. Injection port temperatures should be chosen with this in mind. On the other hand, $Cr(hfa)_3$ is unusually stable, resisting decomposition at temperatures up to 375 °C. It can easily be steam distilled, and distills unchanged from boiling aqua regia or concentrated sulfuric acid (191).

H(fod).-Heptafluorodimethyloctanedione boils at 33 °C (2.7 Torr) and is soluble in most organic solvents. Its complexation reactions are similar to those of the other β-diketones, but it can provide additional latitude in chelation-gas chromatographic analyses. Where volatile chelates containing a given metal are formed by several ligands, the H(fod) and H(tfa) chelates of Cr(III) and Al(III) being typical examples, the vapor pressures of the H(fod) chelates are slightly lower than those of the H(tfa) chelates. However, the primary utility of H(fod) lies in its capability of forming chelates with metals with which no other ligands can form useful complexes.

Anhydrous metal complexes which are coordination

saturated with the ligand have been the most satisfactory for this type of analysis. Hydrate formation causes poor solvent extraction efficiencies into organic solvents (175). For example tfa chelates of divalent metal ions such as Mn, Mg, Zn and Ni, which form hydrates to satisfy coordination requirements, are extracted only to the extent of a few percent (177). In addition, the presence of water has been suggested as the cause of the thermal instability encountered in many hydrated chelates (172). Hydrolysis at elevated temperatures is thought to occur.

H(fod) is a much bulkier molecule than H(tfa) or H(hfa). It forms sterically crowded chelates. This crowding results in a decrease in the tendency for a complex to form a hydrate, even though in some cases coordination is incomplete. Anhydrous Co(II) and Zn(II) bis chelates of H(fod) have been formed, extracted, and chromatographed successfully, even though the coordination requirement of six has not been met (172). This illustrates the usefulness of H(fod) chelates for the analysis of divalent metal ions. In another study, the bis fod chelates of Ni(II), Pa(II), Cu(II), and Be(II) were also quantitatively formed and eluted without apparent decomposition (192).

The tris chelates of the β-diketones generally have the most desirable physical properties for gas chromatography. Rare earth ions tend to accept extra donor groups, easily forming complexes with coordination numbers greater than six. Most tris bidentate rare earth complexes therefore are

hydrates (172). These hydrated chelates have been reported to be thermally unstable (174). Hydrolysis yielding the hydroxobis diketonate and neutral ligand is suspected to occur, both at elevated temperatures and in some cases under vacuum at room temperature (172).

H(fod) forms anhydrous and monoaquo tris complexes with trivalent rare earth ions. The monohydrated complexes and anhydrous are interconvertible by a totally reversible process with no hydrolysis (174). This is believed to be possible because the water molecule may be hydrogen bonded to an electronegative site on the ligand, rather than coordinated to the metal ion (174), due to steric crowding in the complex.

Fifteen tris rare earth H(fod) complexes have been successfully chromatographed (174). They were found to be more volatile than any other known compounds of the lanthanide elements. The synthetic procedure, which involves precipitation of the chelate from aqueous solution, leads to the same difficulties as the similar extraction procedure described earlier. The product, a monohydrate, is typically dehydrated at gas chromatographic elution temperatures and separated in an anhydrous form (174). Purification by sublimation must be done carefully, since the chelates decompose at temperatures slightly above 200 °C (172).

Quantitative extraction of $Co(fod)_3$ from aqueous solutions at sub-ppm levels has been described (193). The aqueous sample solution is mixed with portions of NaOH solution and H_2O_2 solution. After the reaction is complete, the resulting

chelate-benzene solution is decanted and the excess H(fod) is back-extracted to prevent electron capture detector poisoning. The efficiency of extraction was found to be highly dependent on the order in which the reagents were added, while variations in pH from 0 to 6.4 had little effect. The relative insensitivity to pH allows the choice of a pH at which the extraction efficiencies of potential interferents are at a minimum. This method has been used to determine Co in vitamin B_{12} in liver extracts at levels as low as 4×10^{-11} g (193).

One disadvantage of the fod chelates for solvent extraction, as compared to tfa chelates, is the longer equilibration time required. For example, a minimum of 4 hr is necessary for reaching equilibrium when extracting Zn and Co (175). This can sometimes be avoided by using direct reaction techniques.

Sievers et al. (192) report an extremely simple direct chelation procedure for forming fod chelates of Al, Be, Cr, Fe, Ga, Hf, In, La, Mn, Ti, Sc, V, Zn and Zr. A solid sample containing the metals to be analyzed is sealed in a glass capillary with the neat ligand. Heat is then applied briefly to speed up the reaction before injection into the gas chromatograph.

A similar direct reaction procedure has been used in the analysis of Co as the fod chelate (193). Nonaqueous samples were placed in a small vacuum sublimation apparatus along with a small amount of HNO_3 and the ligand. After

reacting at 104 °C for 4 hr, the excess ligand was removed and tris-$Co(fod)_3$ was recovered by sublimation. When the same procedure was carried out at 127 °C, bis-$Co(fod)_2 \cdot 2H_2O$ was formed. The reaction temperature is thus critical if one is to avoid the thermally unstable hydrate.

Instrumentation

Injection.-Metal chelates can be injected into the gas chromatograph either as solids or in solution. One type of solid sampler successfully used by Sievers et al. is a bayonet type which crushes sealed glass melting point capillaries containing the sample in the injection port (192). The solid sample injector consists of a hollow stainless steel tube with a plunger which fits inside. To operate the device the plunger is removed, the sealed capillary is placed in the tube and the plunger is inserted all the way, breaking the capillary by forcing it against the beveled end of the device and releasing the sample in the carrier gas stream. This type of sampler was used with the sealed tube microanalysis method described by Sievers (192). Besides applications such as this where the capillary is also a reaction vessel, the solid sampling technique is useful where a large amount of chelate is to be injected, or where the presence of a solvent is to be avoided.

Liquid injection by microsyringe is used more frequently. It is more convenient than solid samplers, which require that the carrier gas flow be interrupted before each injection and that the glass capillary fragments be removed from the injection port periodically.

Another advantage of liquid injection is that, since the

chelate is already dispersed in the solution before solvent volatilization, instantaneous vaporization can be achieved at lower injection port temperatures than would be required if the sample were introduced as a crystalline solid (187). In addition, the last step in many chelation reactions being solvent extraction, the organic extract lends itself to liquid injection. No capillary sealing, with the accompanying risk of sample loss or decomposition, is required. Repetitive sampling can be made much more quickly and conveniently when a liquid sample and syringe are used.

Detectors.-The three most popular gas chromatographic detectors for metal chelates are the thermal conductivity (TC) detector, the electron capture (EC) detector, and the flame ionization detector (FID). The TC detector is the least sensitive of the three, with a detection range for metals extending from 10^{-4} to 10^{-8} g. It is easy to operate and responds to all compounds. It must be used where non-destructive detection is required due to effluent sampling for further analysis.

The EC detector is selective and extremely sensitive to compounds with high electron affinities such as fluorine substituted metal chelates. It is relatively insensitive to hydrocarbon solvents, permitting detection of picogram quantities of a halogenated compound without interference from the tail of the solvent peak. Some reported lower limits of detection with the EC detector are 3.1×10^{-15} moles for Cr, 1.2×10^{-13} moles for Al, and 2.0×10^{-10} moles for Cu (194);

10^{-13} g for Be (181), 10^{-14} g for Cr (67, 68), and 10^{-11} g for Co and Al (176), all as tfa chelates.

Intermediate between the EC and TC detectors in sensitivity and ease of operation, the FID is an excellent choice for many metal chelate determinations. Lower limits of detection with the FID have been reported as 3.7×10^{-10} moles for $Cr(tfa)_3$, 1.7×10^{-10} moles for $Al(tfa)_3$, and 1.6×10^{-9} moles for $Cu(tfa)_2$. The linear response ranges from 10^{-8} to 10^{-4} g for $Cr(tfa)_3$ compared to 10^{-11} to 10^{-9} g for the EC detector (194). Its operational stability is better than that of the EC detector, and it is not poisoned by large amounts of chelate or ligand. In the ppb range the greater sensitivity of the EC detector is required, but above 5 ppm the FID is the method of choice (194).

Serum application.-The application of metal chelate-gas chromatographic analysis to serum has been limited. Savory, Mushak, and Sunderman (68), and Savory et al. (67) have determined Cr concentrations in serum in the vicinity of 40 ng/ml. They report good recovery of Cr added to serum samples, and good reproducibility. Chelation and solvent extraction of Cr in untreated serum yielded no $Cr(tfa)_3$. This was also true of serum which had been partially wet ashed. In contrast to this, Hansen et al. (69) determined Cr in plasma at the 50 ng/ml level using a direct chelation-sealed tube procedure with no attempt to break down the protein bound Cr complexes by ashing. Their work, which included live animal experiments to evaluate chelation efficiency, describes a simple, direct process with a lower limit of detection around 5 ng/ml of serum.

Taylor et al. (112, 113) have determined Be in biological fluids using a sealed tube reaction similar to Hansen's Cr procedure. They, too, found H(tfa) to be an effective direct chelating agent, forming quantitative chelate yields in the presence of the naturally occurring complexing agents present in serum, thus avoiding time consuming ashing and extraction steps. They added Na_2EDTA to untreated blood to prevent clotting and to sequester interfering metals such as Fe in the blood sample. This enables Be concentrations as low as 0.02 µg/ml to be determined.

H(hfa) has not been used successfully in serum trace metal analysis. Hansen et al. (69) in an analysis of Cr in plasma attempted a direct chelate formation reaction using H(hfa) dissolved in hexane. This solution was placed with untreated plasma in a sealed tube and heated. A maximum of 50% of the metal was chelated under optimum conditions. A white layer at the plasma-hexane interface was assumed to be the solid dihydrate of H(hfa).

Hansen et al. (69) used a modified direct chelation procedure to determine Cr in serum using H(fod). Only 50% of the Cr was chelated, however. H(fod) is a useful complement for H(tfa) in that it forms volatile chelates with metals with which H(tfa) does not. The lower volatility of the fod chelates, however, makes H(tfa) the ligand of choice where possible.

Neutron Activation

Neutron activation analysis (NAA) has been used frequently

as an analytical tool for trace element analysis of serum samples. The general applicability of the technique at the trace level is illustrated by the lower limits of detection listed in Table 9 (116). These figures are based on the measurement of 40 disintegrations per second in a sample irradiated at 5×10^{13} n cm^{-2} s^{-1} for an optimum time period. NAA has the advantages of multielement capability, low detection limits, wide dynamic range, and invariant sensitivity with the nature, size and concentration of the sample. The sensitivity is determined by fixed nuclear parameters such as half-life, cross section and natural abundance, which are constant under all conditions.

In complex samples, the small photopeak from a trace constituent is often partly or completely obscured by γ-rays from other radionuclides. If the half-lives of the offending radioisotope and the radioisotope of interest differ sufficiently, partial photopeak resolution can be obtained by carefully choosing the duration of neutron irradiation and decay. This technique is used in nondestructive, or instrumental NAA. If this approach cannot be used, or if the photopeaks cannot be resolved by a high-resolution detection and counting system, a chemical separation step must be included.

Instrumental neutron activation analysis.-If the difference in half-life between the radionuclide of interest and an interferent can be exploited, separation is not necessary. Short half-life isotopes are measured using irradiation times too short to allow interferent activity buildup,

Table 9. NAA Lower Limits of Detection

E	ppm	E	ppm	E	ppm
Al	0.0002	In	0.000005	Ru	0.0008
Sb	0.0014	I	0.00008	Sm	0.0008
As	0.0016	Ir	0.000001	Sc	0.00006
Ba	0.004	Fe	0.1	Se	0.00006
Bi	6	La	0.0004	Si	0.1
Br	0.00004	Pb	0.1	Ag	0.000006
Cd	0.01	Lu	0.00008	Na	0.001
Ca	0.01	Mn	0.00004	S	0.1
Ce	0.18	Mg	0.01	Sr	0.016
Cs	0.006	Hg	0.002	Ta	0.0012
Cl	0.0008	Mo	0.008	Te	0.004
Cr	0.02	Nd	0.006	Tb	0.002
Co	0.000006	Ni	0.04	Tl	0.004
Cu	0.001	Nb	0.00012	Th	0.00008
Dy	0.000001	Os	0.002	Tm	0.016
Er	0.008	Pd	0.002	Sn	0.014
Eu	0.00001	P	0.08	Ti	0.012
Gd	0.0012	Pt	0.0018	W	0.0018
Ga	0.002	K	0.04	U	0.0004
Ge	0.006	Pr	0.0012	V	0.000016
Au	0.0004	Re	0.0004	Yb	0.002
Hf	0.006	Rh	0.000001	Y	0.018
Ho	0.0004	Rb	0.004	Zn	0.0012
				Zr	0.1

while analyses of longer half-life radionuclides incorporate a delay after irradiation during which a short half-life interferent decays to an insignificant level. Long and short half-life isotopes can sometimes be counted together by using a long irradiation, a decay period, and a short reir-radiation followed immediately by counting.

The most pervasive interference in NAA of biological materials is due to 15-hr ^{24}Na. The parent isotope, ^{23}Na, has a large natural abundance, a high thermal neutron cross section, and is present at concentrations from 10^3 to 10^5 times greater than the trace elements of interest. These factors combined with the relatively high energy of the emitted γ-rays prevent the measurement of small amounts of radionuclides in many biological matrices.

A high resolution γ-ray spectrometer is essential for performing multielement instrumental activation analysis. Coaxial Ge(Li) detector resolution is 10 times better than that of NaI(Tl) detectors. Planar Ge(Li) detectors have still another order of magnitude of improved resolution, although they suffer from a lack of sensitivity due to their smaller active volume. The higher sensitivity of the coaxial detector compensates for its lower resolution, making it the detector of choice for activation analysis. Although NaI(Tl) detectors are 10-20 times more sensitive than Ge(Li) detectors, this does not compensate for their extremely poor resolution.

A high resolution multichannel pulse height analyzer with several thousand channels is needed to take advantage

of the resolution of the Ge(Li) detector when a large energy range spectrum is being recorded. Where possible, direct computer interfacing is also desirable in order to facilitate data handling and reduction.

In spite of interference problems, successful instrumental determinations in biological matrices have been reported. Nadkarni and Morrison (195) described a nondestructive NAA procedure for trace element analysis capable of yielding data for 36 elements in certain types of biological matrices. Irradiation periods ranging from 1 min to 80 hr, with decay periods ranging from 1 min to 4 weeks were used. Due to the extremely low concentrations present, however, the number of elements which can be determined in blood by this method is not as large (195). Blood analysis with short irradiation periods (196, 197), and long irradiation periods coupled with Ge(Li) anticoincidence counting (198) and an elaborate instrumental setup including a filtered reactor neutron spectrum and triple coicidence counting with a $^{56}Mn/^{24}Na$ activity ratio of 700 (197) have been reported.

Yule (199) has published a list of instrumental detection limits in various matrices. Table 10 lists his estimates for blood. Comparison with Table 1 shows that most elements whose serum concentrations are known are present at levels too low to be detected by NAA without first removing the sodium. Comparison with Table 9 shows the decrease in detection limit achieved in blood samples by chemical separation.

Destructive activation analysis.--Advances in the resolution of detectors and multichannel analyzer resolution have

Table 10. Limits of Detection in Blood by Nondestructive NAA

E	ppm	E	ppm	E	ppm
Ag	0.6	Ho	0.4	Re	4
Al	-	I	4	Rh	4
As	4	In	0.1	Ru	2
Au	0.002	Ir	0.006	S	6000
Ba	12	K	1000	Sb	0.08
Br	-	Kr	21	Sc	0.04
Ca	200	La	0.05	Se	4
Cd	500	Lu	0.006	Si	500
Ce	2	Mg	800	Sm	0.04
Cl	-	Mn	0.9	Sn	300
Co	-	Mo	2	Sr	40
Cr	3	N	-	Ta	0.1
Cs	0.4	Na	-	Tb	0.2
Cu	40	Nb	1000	Te	3
Dy	0.06	Nd	6	Ti	200
Er	20	Ne	500	Tl	300,000
Eu	1	Ni	8000	Tm	2
F	30	Os	30	V	0.8
Fe	300	P	1000	W	2
Ga	300	Pb	200,000	Xe	8
Gd	60	Pd	3	Y	10
Ge	50	Pr	8	Yb	0.2
Hf	0.2	Pt	3	Zn	2000
Hg	20	Rb	7	Zr	20,000

greatly reduced the degree of radiochemical purity required from radiochemical separations. In many cases, a group separation is all that is required. Nevertheless, high separation factors are still needed if a very large excess of interferent is present, as is the case with sodium in serum.

Meinke (200) has compared pre- and postirradiation separations. The choice depends on the nature of the sample and the separation technique involved. Postirradiation techniques permit carrier use and require no blank correction, while preirradiation separation is necessary whenever the removed interferent would have resulted in too high a radiation level in the sample.

Ashing.-Most chemical separation procedures require that all atoms of each species, including the carrier, be in ionic form, in aqueous solution, and in the same chemical state. In matrices such as serum, where the trace elements are combined in complex molecules, complete breakdown of the sample is required prior to separation. Ashing is thus considered necessary in most cases.

Trace element losses during wet ashing and high temperature dry ashing have been thoroughly explored (201). The 700-800 °C temperature required for dry ashing in a furnace results in the loss of volatile metals and metal compounds, such as chlorides. Wet ashing, while using more moderate temperatures, introduces impurities via the reagents. The temperatures reached in a low temperature dry asher of the

rf-excited oxygen plasma type can be kept as low as 120 °C. No reagents are required. The technique is slow, but if large sample surface areas are exposed ashing can be completed in a few hours.

Precipitation.-One of the oldest classical techniques, precipitation is frequently used with NAA. The comprehensive group separation schemes of Robertson (202), and the analysis biological samples by Bowen (203), Heydorn and Damsgaard (204), and Rakovic (205) illustrate its usefulness. Special applications of precipitation to sodium separation from biological samples include coprecipitation of sodium on $KClO_4$ (206-208) and precipitation of NaCl in n-butanol saturated with HCl (209).

In general, precipitation is not selective due to coprecipitation and absorption. Large quantities of carrier are required, thus ruling out preirradiation separation and complicating the use of precipitation with other techniques.

Distillation.-One of the best techniques for obtaining high purity separated fractions is distillation. Elements can be distilled as halides, oxides, hydrides, and sometimes even in the elemental state (210). It has been used in NAA as a preliminary step in complex schemes (211, 212), for various biological samples (213, 214), and for trace analysis in blood (38, 57). Compounds for which distillation is applicable are also amenable to more powerful separation techniques such as gas chromatography. Distillation, then, often does not fully exploit the existing potential for efficient separation.

Electrochemical methods.-Controlled potential electrodeposition is a somewhat selective separation technique useful for concentrating elements from large volumes of dilute solutions. It has been used for preirradiation (156, 215) and postirradiation (10, 216) separations in urine (216) and serum (10) analyses. However, since large volumes of serum cannot be drawn from an individual, the potentially extensive preconcentration of this technique cannot be achieved. The selectivity is useful for sodium separation if high decontamination factors can be obtained, but such group separations for counting with high-resolution detectors are better done using another technique.

Electrophoresis has been used for NAA separations (217-220). Copper in serum has been determined this way (220). Since metals in serum can be separated as their naturally occurring complexes, this technique may prove to be of great value in future serum trace element determinations. The restrictions of microgram sample size and reduced selectivity associated with electrophoretic focusing do not seriously affect serum determinations (221).

Dialysis.-During the dialysis of a serum sample all unbound ions, including all the Na^+, are removed. Protein bound concentrations rather than total concentration are thus obtained. Kanabrocki et al. have determined nondialyzable manganese and copper levels in saliva (222), urine (223), cerebrospinal fluid (224), and serum (14, 15). Cotzias et al. (61) have shown that serum proteins will combine with manganese

in the dialysis solution and extract contaminants from the dialysis bag, thereby yielding results which are too high. The labile nature of the serum complexes, which can result in figures being low as well as high, requires that dialysis results be viewed with caution. Olivares (225) combined NAA and dialysis to determine the serum concentration of the metal-containing complex selenocystathionine. In cases such as this, where it is the complex itself which is of interest, dialysis is quite useful.

Gel filtration.-Serum trace elements can be separated as their naturally occurring protein complexes using gel filtration (226). NAA determinations thus obtained include protein-bound I (227), and As, Ag, Au, Cr and Sb (228). The values reported are semiquantitative and do not allow for complex dissociation due to dilution with eluent.

Szilard-Chalmers effects.-Radiochemical separations using Szilard-Chalmers reactions take advantage of metal-ligand bond cleavage in chelates during (n,γ) reactions for isotope enrichment (229, 230). When applied to biological samples, enrichment factors as large as 10 for elements in dried blood and liver have been obtained (231, 232).

Separation techniques such as electrophoresis, dialysis, gel filtration, and paper and thin layer chromatography require that the complicated protein complexes in which the trace elements are bound remain intact for postirradiation separation. Annealing to repair the extensive damage from Szilard-Chalmers reactions must accompany such separations. Little

work on annealing protein complexes has been published, although Szilard-Chalmers effects have been noted (233). The annealing of β-diketonates has been discussed at length (234-239). Heat and gamma-radiation do some repair in the reactor during irradiation, but the greatest metal retention is obtained by heating after irradiation. Plots of retention versus annealing time at constant temperature reach plateaus, the heights of which are temperature dependent (237). At higher temperatures retention quickly falls off due to decomposition (237). Szilard-Chalmers reactions, then, are both useful for some types of separation and a hindrance which must be compensated for in other separation procedures.

Solvent extraction.-Highly selective extraction procedures have been reported for use in radiochemistry and other applications. The systems available are quite numerous. Books are available which discuss fundamental principles as well as specific methods (240, 241). Several areas which have received recent attention may be useful for trace element analysis in serum. Solvent extraction of metal chelates such as the β-diketonates discussed earlier, 8-mercaptoquinoline (22), and thenoyltrifluoroacetone (18, 242-245) is one such area. Another is the use of liquid ion exchangers such as di-(2-ethylhexyl)orthophosphoric acid (246). Liquid ion exchangers combine the speed of solvent extraction with the selectivity of ion exchange. Direct elemental extraction without complexation has not received much attention, although it has been reported (247).

NAA with solvent extraction has been used with biological samples including serum, with reported sodium decontamination factors of 10^4 (248). One major obstacle is that extraction conditions which allow extraction of a large number of elements often do not result in adequate sodium removal. For this reason, where both multielement analysis and high decontamination factors are needed, solvent extraction is often found as one of a series of separation steps.

Inorganic separations.-Rapid, selective separations can be accomplished by passing sample mixtures over columns of various inorganic materials. Several possible reaction mechanisms exist: isotope exchange, redox reactions, ion exchange, and mixed crystal formation by recrystallization.

In reviews of this topic (249, 250), Girardi reports both large decontamination factors and lower sample capacities than for organic ion exchangers. A survey of the NAA applicability of over 3,000 adsorption experiments on various ionic precipitates lists hydrated metal oxides among the more useful materials (251, 252). Hydrated MnO_2 retains numerous ions completely (253), while hydrated tin oxide, SnO_2, is mainly useful for ^{32}P retention. Hydrated antimony pentoxide (HAP) in highly acidic systems has been shown to remove only Na and Ta from a mixture of 60 elements (254). HAP and SnO_2 have been used together for Cr determination in serum by NAA (64).

The high selectivity and efficient sodium decontamination have been matched by isotopic exchange with a column of NaCl crystals using an organic eluent (255). The technique is

rapid and selective, with sodium decontamination on the order of 10^8 and neglible retention of most other elements. Magnesium removal from biological matrices with a decontamination factor of 10^3 using $MgNH_4PO_4 \cdot 6H_2O$ (96) illustrates extension of the principle where suitable eluents and solid forms of the element involved are available.

Amalgam exchange with liquid mercury has been used by Kim and Silverman to determine mercury in various matrices (256, 257). Mercury has been determined in whole blood using this technique (258, 259). Amalgam exchange has been used to remove radioactive Bi, Cd, In, Pb, Sr, Ti, Zn and Cu from aqueous solutions (260, 261). Equilibrium is attained quickly, and excellent decontamination can be achieved at trace levels, making this technique suitable for trace element analysis in serum. Unfortunately, one can work best with only one element at a time. Moreover, the technique is applicable to only a limited number of elements.

Ion exchange.-The use of ion exchange resins in NAA is well established, with many routine procedures for both selective individual separations and sequential group separations having been reported.

Ion exchange material can be divided into four groups: polymeric resins, celluloses such as carboxymethyl cellulose, inorganic exchangers such as zirconium phosphate, and liquid exchangers such as dinonylnaphtalene sulfonic acid (262). Of these, the resins are the most widely used cation exchange material, with advantages including high capacity, hydrophilic

gel structure with easily reached functional groups, high resistance to degradation by acids and alkalies, and relatively unchanging ion exchange properties (263). There are many different kinds and brands of resin available. For many separations the kind of resin used is not important. Those with the same functional group, often $-SO_3H$, will behave similarly. One useful property of sulfonic acid exchangers on a polystyrene base is that these resins are much more resistant to radiation damage than the anion exchange resins usually employed (262).

Chelating ion exchangers differ from ordinary ion exchangers in three respects. The first is higher selectivity due to dependence on the chelating group rather than ion size, charge, or other physical properties. The second difference is that the bond strengths are greater, 15-20 kcal/mole, as compared to 2-3 kcal/mole for electrostatic bonds in ordinary resins. The third difference is kinetic. The chelating resin exchange processes are slower, controlled by either particle diffusion or second order chemical reactions (264). The strong bond between the metals and the chelating resins permits their extraction from very dilute solutions of high ionic strength (264), such as seawater (265) and dissolved geological samples (266). Copper has been separated from biological matrices in this way (267). This property also allows the extraction of complexed metal ions from a complexing medium. Metal ion removal from protein complexes such as those in serum has not been reported.

There are many excellent publications describing ion exchange theory and technique. Massart's monograph (262) deals specifically with radiochemical cation separations and discusses both the selectivity and kinetics of ion exchange separations. Resin characteristics such as capacity (263), particle diameter (268), cross linkage, porosity (269) and the effect of temperature on HETP (270) have also been treated.

Special techniques dealing with matrix separation in trace NAA have been developed. Precipitation ion exchange, which uses the insolubility of matrix metal chlorides such as NaCl in the 12M eluent, is one such method (271). It works well for matrices containing large amounts of Na, Ag, Ba, K and Sr. Another technique for matrix activity removal uses isotope exchange on a resin in a stable form of the cation. Breakthrough of the radioactive matrix is delayed while the radioactive trace elements are rapidly eluted (272).

Ion exchange resins lend themselves to automated multi-element separations because the eluate from one column can already be in a form suitable for introduction into the next column. Systems using many columns in complex, branched separation schemes have been described (273-282). Ricq (273) has explored the use of superimposed columns, while Moiseev et al. have described a system for determining 27 elements at trace levels in a silicon matrix (274). Similar systems designed for biological samples have been developed by Comar and LePoec (275). Samsahl has described automated multiple column ion exchange and solvent extraction group separation systems for biological samples allowing determination of up

to 40 elements (276-278). Speed is emphasized (277-279), as little as 5 min being required for the processing of four samples (278). Of the numerous other automatic and semi-automatic group separation schemes developed, that of Morrison et al. (280, 281) is perhaps the most extensive and analytically useful. It enabled measurement of 41 trace elements in the Apollo 11 lunar samples by NAA after separation into six groups using ion exchange, solvent extraction, and HAP extraction. This degree of radiochemical purity is sufficient with high-resolution counting equipment (282).

NAA with ion exchange separations has been used for the analysis of many different types of biological samples (225, 265, 283-287). Determination of various elements (5, 38, 57, 83), including I (76, 284-290) using iodine-specific resins (289) and a sequential group separation scheme for 12 elements (290) have been reported. Ion exchange membranes have also been used in biological analyses (291). The characteristics of ion exchange resins, especially chelating resins, strongly suggest their application to serum trace element analysis by NAA.

Paper chromatography.-Various chromatographic techniques for postirradiation separation in NAA have been reported, but separation before irradiation without diluting or disturbing the separated fractions can only be accomplished with paper as the support. The use of paper chromatography in NAA has been reviewed by Budzynski (292). Studies involving separration and activation of metal ions (292-296), complex molecules of biochemical interest (74, 297-302), and possible

interferents in the chromatographic paper (303) have been reported. The number of elements involved in reported separations varies, with 7 (293, 294), 26 (295), and isolation from mixtures of 40 or more (296) being representative. The techniques used range from ordinary procedures (77) to the use of columns consisting of stacked ion exchange paper disks which provide sodium decontamination factors of 10^4 (304).

Molecular separations in serum analysis offer an additional dimension for trace element metabolism study. Biological molecules or chelates of trace elements which lack atoms with desirable activation characteristics can be determined using derivative activation chromatography (297-300). Serum molecules such as phospholipids (292) and iodine-containing thyroid hormones (74, 226), and protein complexes of activating metals yield quantitative and qualitative data for both the element activated and the protein complex (292). This is possible because the unique preirradiation separation capability eliminates adverse effects from Szilard-Chalmers decomposition.

For determinations above the trace level, interference from impurities in the paper is not significant (74, 303). The only major contaminants found are Na, Cl, and Mn. These can be effectively reduced by washing the paper. However, due to the tubular structure of the paper fibers, all interferents cannot be completely removed, thus jeopardizing the accuracy of trace determinations (292). Other drawbacks include deterioration of the paper in the reactor and lack of resolution of complex mixtures due to inhomogeneity of the paper.

The advantages of thin layer chromatography over paper chromatography include greater speed and more selectivity in the stationary phase. Separation of metal ions has been reported (305, 306), but the application of thin layer chromatography to NAA has not been explored, probably due to the lack of feasibility of preirradiaiton separation.

Gas chromatography.-Gas chromatographic separations are rapid, highly efficient, and can be carried out without the use of carriers. In addition, these separations can be optimized with respect to time, resolution, or sample size. This allows the application of the technique to short-lived isotopes, multicomponent mixtures and trace constituents. These separations are very useful in trace analysis since they in effect result in infinite resolution in the γ-ray spectrum. This allows the lowest limits of detection to be reached using NaI(Tl) detectors.

Gas chromatography has been used fairly extensively in radiation chemistry (307), but the application of the technique to the separation of photon-emitting radionuclides in neutron activation analysis has been reported infrequently. The application of gas chromatography to NAA was first reported by Cram and Brownlee (308-310). They demonstrated the usefulness of gas chromatography for the separation of short-lived radioisotopes in high speed postirradiation separations, some as fast as 5 s, allowing measurement of 10.7 s ^{20}F.

The use of metal chelates extends the usefulness of gas chromatography to encompass many trace elements. As stated

in the Gas Chromatography section, β-diketonates offer the greatest potential, allowing more than three-quarters of the elements in the periodic table to be separated and eluted easily, quickly, and with high resolution. The inherent speed and selectivity of gas chromatography combined with the desirable physical properties and ease of synthesis of the metal chelates make the technique an excellent prospect for NAA separations.

The use of a high-resolution γ-ray spectrometer with a gas chromatograph constitutes the ultimate in specific detection for NAA, while the use of efficient NaI(Tl) scintillation detectors allows the lowest limits of detection to be reached.

CHAPTER II

EXPERIMENTAL

Physical Facilities and Equipment

Nuclear Reactors

UFTR.-The University of Florida Training Reactor (UFTR) is an Argonaut type, graphite reflected and water moderated, with 100 kW thermal maximum power. It was used in all the short irradiation, short decay experiments because of its close proximity to the separation and counting equipment. Flux data for the port used in this work appear in Table 11.

GTRR.-The Georgia Tech Research Reactor (GTRR) is a CP-5 type, heterogeneous, heavy water moderated and cooled, fueled with highly enriched plates of aluminum-uranium alloy. Higher thermal neutron flux combined with longer daily operation hours made the GTRR more useful for long irradiations. Flux data for the ports used in this work appear in Table 11.

Separation Equipment

Low-temperature dry asher.-Ashing was carried out using a model LTA-600 asher, Tracerlab-Richmond, Richmond, California. Ashing times of 4-12 hr at an rf power level of 200 W were used.

Gas chromatograph.-A Varian Model 1200-1 gas chromatograph with a hydrogen flame ionization detector, a linear

Table 11. Irradiation Facilities

	Georgia Tech Research Reactor	University of Florida Training Reactor
Power Level	1 MW	100 kW
Cd Ratio	2000:1	1:1
Gamma Flux, R/hr	1×10^{6} R/hr	2.4×10^{6} R/hr
Thermal Neutron Flux, n cm^{-2} s^{-1}	GTRR V-21 2×10^{13} GTRR V-37 7×10^{11} GTRR V-43 5×10^{11}	Center Vertical Port 1.8×10^{12}

temperature programmer, and a 1/4-in injection port was used in these studies. The system was all glass and Teflon to eliminate reactive surfaces on which the chelates could decompose. The column tubing was nominal 1/8-in o.d. (0.095-in i.d., 0.016-in wall thickness) Teflon (Alpha type, TFT-250/11). The injection port liner was a 6-mm (approximately 0.25 in) o.d. by 14.5-cm Pyrex tube with the downstream end constricted to give a gas tight seal with the 1/8-in o.d. Teflon column forced into the constriction. A smaller 7.5-cm by 3-mm o.d. Pyrex liner fit inside the larger tube. One end of the liner was tapered to fit snugly inside the Teflon column 5 mm from the packing material. It was this liner which was removed for γ-ray counting.

The instrument was also equipped for injecting solid samples. For this type of injection the injection port liner was replaced by a solid sampling device. It consisted of a stainless steel tube and mating plunger which broke sealed 2-mm by 2-cm capillaries containing the sample inside the injection port in a flowing He stream (192).

No Swagelok fittings were used. Instead, the Teflon tubing was forced inside pieces of tapered Pyrex tubing to give a tight fit. The Pyrex was tapered from both ends, allowing the two Teflon pieces to touch each other, thus giving zero dead volume. Liquid injections were made with a 10-μl Hamilton syringe.

For chromatographic peak area measurements, the electrometer output was connected to an Infotronics Model CRS 100

Digital Readout System digital gas chromatographic peak integrator with Victor printer.

Vapor-phase separation apparatus.-The first vapor-phase separation apparatus was simply the gas chromatograph with its column replaced by an empty 6-in piece of 1/8-in o.d. Teflon tubing. A hole in the oven wall allowed a 10-cm long, 5-mm o.d. Pyrex tube to extend from the room temperature atmosphere of the laboratory into the oven, where it was attached to the empty Teflon tube. Sufficiently volatile injected samples could thus pass through the system and condense in the Pyrex tube. A 100-µl syringe with a Teflon-tipped plunger (Precision Sampling Corp., Baton Rouge, Louisiana) was used for injection.

The improved vapor-phase separation apparatus did not use the injection port for sample introduction. Instead, the 6-in Teflon tube in the first apparatus was replaced by a horizontal shallow "U-shaped" Pyrex tube, 8 cm long, 1 cm o.d., with a depression at the bottom to hold the chelate solution (Figure 2). Both the inlet and the outlet arms were horizontal. The He flowed through the tube carrying vapor from the mixture into the cooler Pyrex tube for trapping.

Counting Geometry

Three different types of counting geometry were constructed and their usefulness in counting chromatographically separated radioactive metal chelates evaluated. Except for the Pyrex traps, the effluent reached the counting geometry configurations via a 1/8-in o.d. Teflon tube enclosed in a 1/4-in o.d.

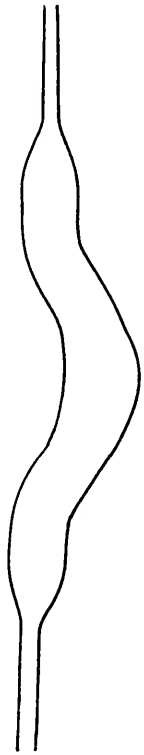


Figure 2. Vapor-phase Separation Apparatus

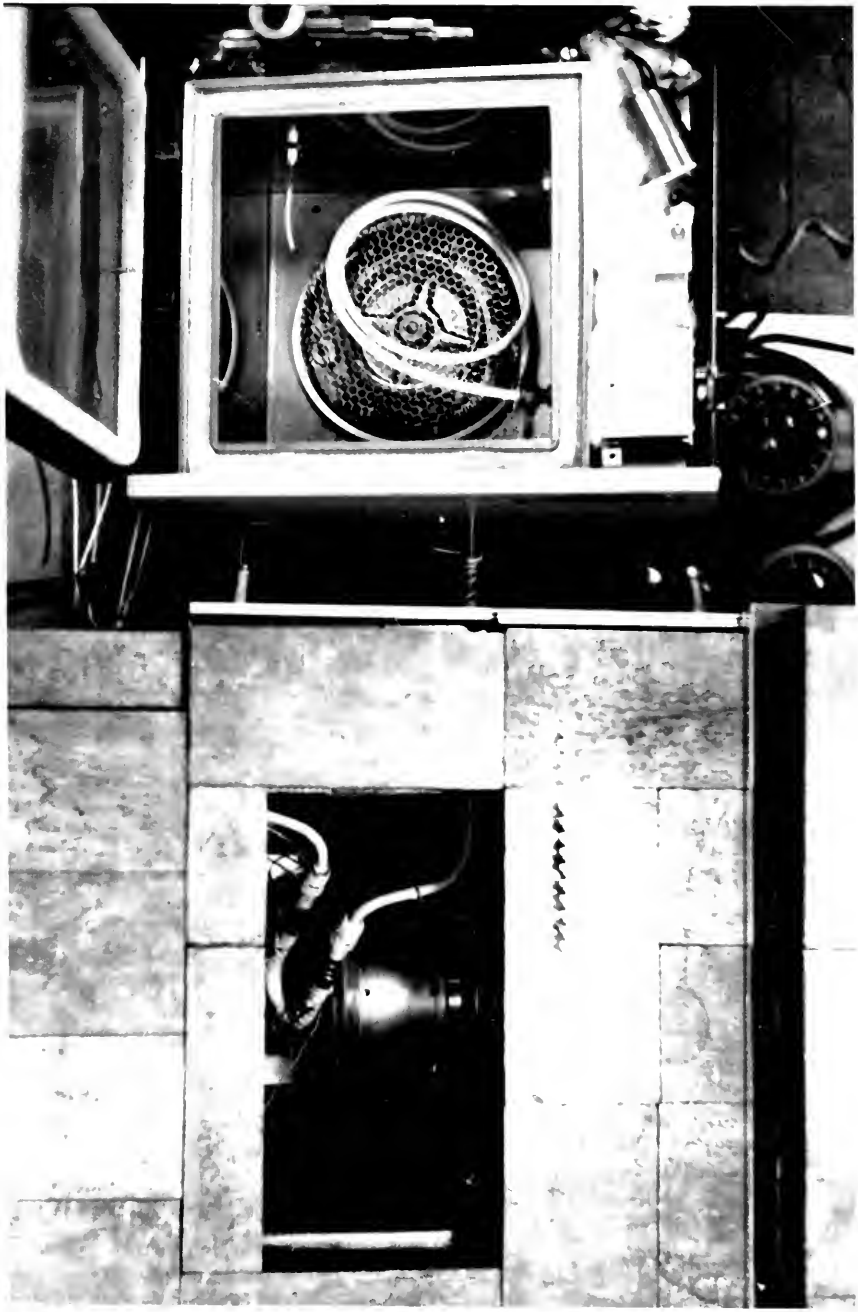
copper tube wrapped with heating wire and maintained at the injection port temperature. The tube passed through the oven wall and into the lead cave beside the instrument. This tube, the gas chromatograph, the lead cave and the improved reversible counting geometry are shown in Figure 3.

Stainless steel spiral.-The stainless steel spiral is a flow-through type counting geometry. The radioactive sample is not trapped; it flows past the detector without stopping. The counting time in flow-through geometries can be increased by forming the tube into a spiral. The length of tube in front of the detector is increased, and the sample is in front of the detector for a longer period of time. If even more counting time is required, the carrier flow can be stopped and the entire separation and elution process temporarily interrupted. Under most circumstances, this has no deleterious effects on the separation of components still on the column.

The spiral was constructed from a 57-in long piece of 0.125-in o.d., 0.005-in wall thickness 316 stainless steel tubing (Superior Tube Company, Norristown, Pennsylvania). It was wound in a flat spiral with an outside diameter of 2.9 in and an inside diameter of 1.1 in. The spiral was wrapped as a unit with heating wire and asbestos tape. A thermocouple was placed inside at the center of the spiral.

Charcoal traps.-The charcoal traps were constructed from 10-cm long, 5-mm o.d. Pyrex tube tapered at one end to fit tightly inside the Teflon tube. A 1-in section of the trap was packed with 30-50 mesh activated charcoal. The

Figure 3. Gas Chromatograph and Lead Cave, with Column, Heated Transfer Line, Reversible Counting Geometry, NaI(Tl) Detector and Exit Line.



inlet to the trap was located inside the column oven. The rest of the tube extended through a hole in the oven wall. The outlet and most of the charcoal were at room temperature. A 5-in piece of thin-walled stainless steel tubing packed with a 1-in bed of 40-50 mesh activated charcoal was also used.

Reversible counting geometry.-A simple reversible counting geometry and a more refined version were constructed. The simple geometry consisted of a 6-in long, 0.25-in o.d. piece of Teflon tubing packed with 2.5 in of 20% SE-30 coated on acid washed, DMCS treated Chromosorb P. This tube was enclosed in a larger aluminum tube wrapped with heating wire. Cool air could be passed around the Teflon tube inside the aluminum tube to cool the packing prior to trapping. Purge He could be introduced at the upstream end to empty the geometry while it was being heated by heating wire.

The improved geometry, Figure 4, had a better design than the geometry described above. A 1-in long segment in the center of a 13-in long, 0.25-in o.d. Teflon tube was packed with 15% SE-30 on 80-100 mesh High Performance Chromosorb W (Hewlett-Packard). The packed region was located at the midpoint of a 1-in radius 180° bend. The center of curvature of the bend coincided with the axes of the two cylindrical NaI scintillation detectors. This design allowed a radioactive sample to give the same detector response at every point along the bend.

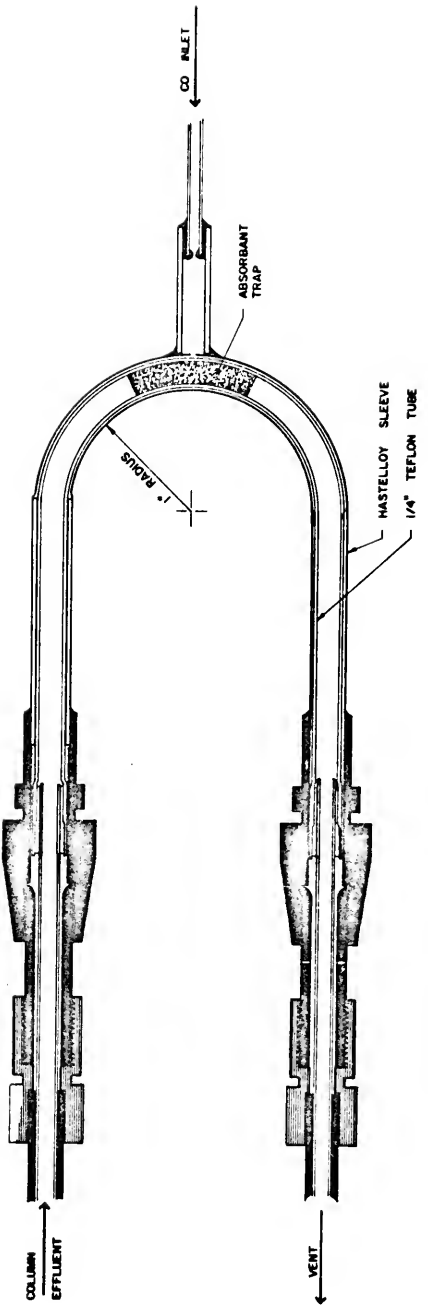


Figure 4. Improved Reversible Counting Geometry

The Teflon tube and packing material were enclosed in a 0.332-in o.d., 0.0125-in wall seamless Hastelloy tube (Superior Tube Company, Norristown, Pennsylvania). The Hastelloy had special brass fittings on each end which enclosed 1/4-to 1/8-in unions made of Pyrex. They joined the 1/4-in Teflon geometry tube and the 1/8-in Teflon inlet and outlet tubes. The brass fittings joined the Hastelloy and the 1/4-in copper tubes which enclosed the 1/8-in Teflon tubes. The Teflon was enclosed in metal to ensure uniform heating of the system to prevent chelate condensation or decomposition. The metal tubes were wrapped with asbestos-insulated #24 Chromel heating wire. The entire system was wrapped with asbestos tape to provide additional insulation and thermal stability.

Carbon dioxide with a pressure of 20 psi could be forced through a 0.025-in orifice into the space between the Hastelloy and the 1/4-in Teflon tube at the 1-in packed region to cool the packing. This created a large thermal gradient along the packing material. The inlet was operated at 150 °C, while the outlet was operated at 60 °C. The chelate is trapped by the 90 °C gradient in the packing. The CO_2 was vented through the outlet arm of the geometry. A special sleeve in the inlet arm prevented CO_2 from being forced back and cooling the inlet. The inlet and outlet heat were controlled independently, allowing inlet heat for trapping, no heat for γ-ray counting, and both inlet and outlet heat for purging.

The He flow network between the two-stage pressure regulator and the injection port was modified to allow rapid

shutoff of the carrier gas supply to the column for stop flow experiments. This was done with a normally open electric valve (Skinner type V15DA1125V09). A normally closed electric valve (Skinner type V52DA1125V18) and a precision metering valve (Nupro, Inc.) were installed to allow bypassing the column with carrier gas to purge the counting geometry and its connecting tubing with a carefully regulated He flow.

Counting and Data Processing Equipment

High-resolution γ-ray spectrometer.-A 50-cc active volume, wrap-around coaxial design, high resolution lithium-drifted germanium detector (Nuclear Diodes, Prairie View, Illinois) was used. The manufacturers specifications were as follows: resolution 2.3 keV at 1.33 MeV; peak-to-compton ratio 23:1; efficiency relative to 3 x 3 sodium iodide detector 12.5%.

An Ortec Model 456 high voltage power supply (Ortec, Oak Ridge, Tennessee) was used to bias the detector at 2500 V. The output from a Nuclear Diodes Model 103 preamplifer and an Ortec Model 451 spectroscopy amplifier was digitized by a Wilkinson type analog-to-digital converter, Northern Scientific Model NS 629 (Northern Scientific, Middleton, Wisconsin). The ADC was capable of 8192 channel resolution and operation at a 50 MHz clock rate.

The ADC was connected through an interface to a PDP-8/L minicomputer (Digital Equipment Corp., Maynard, Massachusetts) with 8K of available core storage. The 12 bit words, when

double precision storage was used in the upper 4K of core, allowed the use of 2048 channels, each with a 16,777K count capacity.

Input-output devices used with the computer system included a Model ASR-33 teletype (Teletype Corp., Skokie, Illinois), an ITT Model 1935D 15-in display Oscillscope on which spectra were displayed, a Hewlett-Packard Model 7127A strip chart recorder with a multiple range input module (Hewlett-Packard, Pasadena, California) with which the spectra were plotted, and a Tri-Data Model 4096 magnetic tape cartridge unit with which all bulk storage, both spectra and software, was done.

The interface and software for the spectrometer system were designed and assembled by David B. Cottrell. A more complete description of the system can be found elsewhere(311).

NaI(Tl) scintillation spectrometer.-Two detectors were used. Each was a 3-in by 3-in thallium-activated sodium iodide crystal with mating multiplier phototube (Harshaw Integral Line Detector, Nuclear Chicago Model 10-9, Nuclear Chicago Corp., Des Plaines, Illinois) connected to a scintillation preamplifier (Nuclear Chicago 10-17) and spectroscopy amplifier (Nuclear Chicago Model 27001). The single channel analyzer (Nuclear Chicago Model 27352) output was counted with a preset count six decade electronic scaler (Nuclear Chicago Model 27104) for gross γ-ray measurements.

The γ-ray spectra were recorded using a 400-channel Nuclear Chicago RIDL 34-27 Series Scientific Analyzer System

with a Model 30-35 Four Input Mixer/General Purpose Amplifier, a Model 23-4 Analog-to-Digital Converter with Buffer Register, and a Model 52-57 Deluxe Display Control. Also included in the system were a display oscilloscope (Nuclear Chicago Model 52-56), a Teletype (ASR-33, Nuclear Chicago Model 44-29), and a five decade preset timer (Nuclear Chicago Model 27101). Plots of the spectra were made with a Plotamatic 850 X-Y Plotter (Data Equipment Corp., Santa Ana, CA).

Lead shielding.-All of the 400-channel NaI(Tl) scintillation spectra were measured inside a lead cave with 40-in x 12-in x 14-in inside dimensions and 4-in thick walls on the top and four sides. The bottom was 2 in thick. The NaI(Tl) detectors were mounted horizontally with the metal chelate effluent passing between them.

The high-resolution Ge(Li) detector, having lower sensitivity, required less shielding than the NaI(Tl) detectors. It was housed in a cave with 12-in x 16-in x 16-in inside dimensions and 2-in walls on four sides and the top. The bottom was unshielded.

Procedures

Ashing

Serum samples came from a human serum pool drawn and processed in the normal course of activities at the Clinical Laboratories at the J. Hillis Miller Health Center, University of Florida. All glassware with which the serum came in contact before irradiation was cleaned by washing with soap and water,

soaking in Nochromix (a metal-free substitute for dichromate in sulfuric acid, Godax Laboratories, New York, New York) for 12 hr, soaking in concentrated HNO_3 for 12 hr and then leaching in demineralized water for 12 hr. The glassware was rinsed in demineralized water after each step. The serum was freeze-dried in sample boats in the ashing chambers. This was accomplished by freezing the serum with dry ice outside the chamber and reducing the pressure to 1 Torr immediately after placing the frozen serum in the chamber. Ashing periods from 4 to 12 hr at an rf power level of 200 W were used.

Chelation from Solid Ash

Three serum samples were processed using different procedures for separation and irradiation. The first sample was subjected to a simple separation before a long thermal neutron irradiation. The second sample was processed in a similar way, except that the separation scheme was more complex. The third sample was irradiated for a short time prior to separation.

Irradiation.-The solid residue and supernatant liquid from the first serum sample were irradiated together in V-21 of the GTRR for 49.5 hr. The solid residue, supernatant liquid, and blank solution from the second serum sample were irradiated together in V-21 of the GTRR for 42.5 hr. The ash from the third sample was irradiated in the UFTR for 30 min.

Gamma-ray counting.-Counting was done with the high-resolution spectrometry system. The organic layer from the first sample decayed 26 days before counting. The solid

residue decayed 20 days before counting. The organic layer and blank from the second sample decayed four days before counting. The solid residue decayed two weeks before counting. The third sample decayed one hour before counting.

Chelate Extraction from Ash Solution

After dissolving the ashed irradiated serum, carrier was added and the chelates of the trace elements in the aqueous layer were extracted into a benzene solution of the ligands H(fod) and H(tfa), leaving the sodium in the aqueous layer. Purification of the metal chelates in the vapor phase provided still further isolation from sodium. One simple procedure and three other procedures using a complex carrier solution were used.

Carrier preparation.-The carrier used in the simple procedure consisted of 6 mg of $Cs(NO_3)_3$, 6 mg of La_2O_3, 4 mg of $CuCl_2$, and 2 mg of Sm_2O_3. These salts were added to the ash as solids. The makeup of the carrier solution used in the multielement procedures for the other three samples is shown in Table 12. The weight of each compound in the complex mixture was chosen to yield 0.01 mmole of element per milliliter of final carrier solution. The metals and metal salts were dissolved, mixed, and the mixture was diluted to 25 ml.

Irradiation.-Four 6.5-g serum samples were ashed and irradiated separately for 30 min in the UFTR.

Chelation.-Each of the four samples was processed immediately after it was removed from the reactor. The simple carrier procedure used in the first extraction

Table 12. Complex Carrier Solution

As	Hf
Au	Hg
Ba	In
Cd	La
Co	Mn
Cr	Ni
Cs	Pd
Cu	Sc
Dy	Se
Eu	Sr
Ga	Zn

experiment consisted of adding 0.5 ml of a benzene solution containing 50 mg/ml each of the ligands H(fod) and H(tfa) to the irradiated ash, stirring, and allowing the mixture to react for 10 min. The supernatant liguid was decanted. Next, the ash was dissolved in 0.5 ml of 16M HNO_3 and 0.2 ml of deionized water, and the solid carrier mixture was added to the dissolved ash solution. Some solid residue remained after the addition. The pH was adjusted to 6 with 2M NH_4OH. A 0.75-ml portion of the benzene-ligand solution was then added and the mixture was shaken for 30 sec. After allowing 10 min for the mixture to separate, the organic layer was removed. The aqueous portion was washed with 0.2 ml of benzene and all three benzene portions were combined for separation and counting.

A similar procedure with a complex multielement carrier solution was used for the next three ashed serum samples. After irradiation, the sample A ash was dissolved in 0.5 ml of 2M HNO_3, 1 ml of carrier solution was added, and the pH was adjusted to 6 with 2M NH_4OH. One milliliter of the benzene-ligand solution was added, the mixture was shaken, and then it was allowed to equilibrate 5 min. After another shaking and 5 min equilibration the pale blue benzene layer was removed. The pH was then adjusted to 4, the extraction was repeated, and the two organic solutions were combined for counting.

Sample B was processed in nearly the same way. The organic solution was added before the 1-ml carrier addition

and subsequent pH adjustment to 6 with 2M KOH. After all the components had been added, the mixture was centrifuged and the pale yellow organic layer was removed.

The radioactive ash from serum sample C was dissolved in 1 ml of 2M HNO_3 and 1 ml of the carrier solution was added. The pH was adjusted to 6 with 2M KOH and the metal hydroxide precipitate was removed by centrifugation. The supernatant was extracted with 1 ml benzene-ligand solution. The aqueous layer and the precipitate were then recombined and the pH was adjusted to 4 with 2M HNO_3. The precipitate remained 30% undissolved at this point. The solution was extracted with 1 ml of the benzene-ligand solution and the two extracts were combined for counting. The remaining solid was purified by reprecipitation prior to counting.

Vapor-phase separation.-The extract from the first experiment was injected into the first vapor-phase separation apparatus in 30-µl segments 1 min apart. After 0.75 ml had been injected, the oven and injection port temperatures were increased to 200 °C over a 15-min interval. The flow was then stopped and the charcoal trap was removed for counting.

The next three samples, A, B, and C, were processed using the improved vapor-phase separation apparatus. The apparatus was operated for sample A at 100 °C for 7 min, 150 °C for 5 min, 175 °C for 5 min, and 200°C for 10 min. The He flow rate was 20 ml/min. Samples B and C were processed at 100 °C for 3 min, 140 °C for 3 min, 170 °C for 3 min, and

200 °C for 10 min. The He flow rate was 75 ml/min. The purpose of the multiple temperature vaporization was to allow the lower vapor pressure chelates to vaporize without risking thermal decomposition of the higher vapor pressure chelates, which leave the oven before the temperature gets too high.

Gamma-ray counting.-Counting was done with the high-resolution spectrometry system. The radiation from the ash was measured for 1 min prior to solvent extraction. A 4-MeV full scale γ-ray spectrum was recorded. After extraction, and before vaporization, a 2-MeV full scale γ-ray spectrum of the benzene solution containing the chelates was recorded. After separation, several spectra of the charcoal traps containing the radioactive metal chelates were recorded.

Annealing Studies

The extent of Szilard-Chalmers degradation in both treated and untreated thermal neutron-irradiated benzene solutions of some metal chelates was determined. The solutions were injected into the gas chromatograph and the γ-ray spectra of the material trapped at the outlet were compared with the spectra of the material retained by the various parts of the instrument with which the sample came in contact.

Chelates with no treatment.-Three metal chelate solutions were prepared. The first contained 32.5 mg of $Cf(tfa)_3$, the second contained 7.0 mg of $Cr(hfa)_3$, and the third contained 30.0 mg of $Cr(fod)_3$. Each chelate was dissolved in 1 ml of benzene (Nanograde, Mallinckrodt Chemical Works) which had been dried over molecular sieve (13X Linde). The three solutions were irradiated in V-43 of the GTRR for 14.5 hr.

The gas chromatographic columns and conditions used for retention measurement are listed as No. 1, Table 13. They were conditioned overnight at 170 °C and treated with 10 µl of DMCS (Dimethyldichlorosilane, PCR, Inc., Gainesville, FL). The injection port liner was included in the silanization.

The solutions were injected in 5 µl portions 1 min apart. A total of 100 µl each of $Cr(tfa)_3$ and $Cr(fod)_3$, and 150 µl of $Cr(hfa)_3$ was injected. After each of the three series of injections, the carrier gas and oven heat were left on for 30 min before dismantling the system. All counting time periods were 10 hr except for column measurements which were 15 and 20 hr.

Chelates with treatment.-Three treatment procedures were evaluated for rechelating the metal ions which had been separated from their ligands by recoil from the complex after neutron capture. The procedures were irradiation with 10% excess ligand, irradiation with 10% excess ligand followed by annealing (heating), and irradiation with 100% excess ligand followed by annealing. These evaluations were all done with $Cr(tfa)_3$.

Two solutions, one containing 30.2 mg ($Cr(tfa)_3$ and 2.7 mg of H(tfa) (10% excess) in 1 ml of benzene, and one containing 30.4 mg of $Cr(tfa)_3$ and 27 mg of H(tfa) (100% excess) in 1 ml of benzene, were irradiated in V-43 of the GTRR for 14 hr.

After irradiation, 20 injections, each 5-µl of the 10% excess ligand sample, were made into the gas chromatograph. After dismantling the instrument and measuring the radio

activity of its various components, the remainder of the sample was resealed, annealed 9 hr at 100 °C, and the experiment repeated. The same injection and counting procedure was followed with the 100% excess ligand sample after it had been annealed 9 hr at 100 °C beginning immediately after irradiation. All chromatographic conditions were the same as those in the previous section describing chelates without annealing.

Gas Chromatographic Studies

The extent to which various radioactive metal chelates of 1,1,1-trifluoro-2,4-pentanedione [H(tfa)], 1,1,1,5,5,5-hexafluoro-2,4-pentanedione [H(hfa)], and 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione [H(fod)] were quantitatively eluted from a gas chromatograph was studied. Each tfa and fod metal chelate was synthesized from radioactive metal and dissolved in benzene. The hfa chelates were purchased. They were irradiated and injected as solids without any chemical processing or purification. The chelate solution (or solid sample) was injected into the gas chromatograph and the chelate eluted and trapped. After trapping the chelate, the system was dismantled, and the packing was placed in a 60- by 150-mm Pyrex petri dish. Separate radiation measurements of the metal chelate remaining in the solid sampler, injection port liner, column tubing, column packing, and charcoal trap were made. Where solid samples had been used, the quartz capillary fragments in the injection port

were counted and the injection port was rinsed with acetone and the solution was counted.

Materials.-The H(tfa) was redistilled and the 106-107 °C fraction retained. The H(fod) was also redistilled. Both were obtained from PCR, Inc., Gainesville, Florida. The $Cu(hfa)_2$, $Cr(hfa)_3$, and $Mn(hfa)_2$ were obtained from Pierce Chemical, Rockford, Illinois.

The radioactive isotopes used were produced in the UFTR from commonly available reagents, with the exception of the following, which were purchased from New England Nuclear, Boston, Massachusetts: ^{51}Cr in 1M HCl, 0.02 mg/ml, 3.7 mCi/ml; carrier-free ^{7}Be in 0.5M HCl, 4 mCi/ml; and ^{59}Fe in 1M HCl, 0.165 mg/ml, 2.42 mCi/ml. The benzene (Nanograde, Mallickrodt Chemical Works) was dried over molecular sieve (13 X Linde). The system was silanized using dimethyldichlorosilane (DMCS, PCR, Inc.).

Chelate synthesis

$Cr(tfa)_3$.-The radioactive metal chelate was prepared by dissolving 40 mg of $CrCl_3 \cdot 6H_2O$ in 0.6 ml of H_2O. Then 0.9 ml of ^{51}Cr solution, 300 mg of urea, and 86 mg of H(tfa) were added. The mixture was refluxed for 7 hr at 100-115 °C. The solid product was washed with water and air dried on filter paper. It was then dissolved in ether and sublimed at 150 °C. The improved vapor-phase separation apparatus was used for purifying the chelate. Dark purple $Cr(tfa)_3$ crystals weighing 47 mg were recovered and redissolved in 1.5 ml of dry Nanograde benzene for chromatographic injection.

Cr(fod)$_3$.-The radioactive metal chelate was prepared by dissolving 52.5 mg of $Cr(NO_3)_3 \cdot 9H_2O$ and 0.3 ml of $CrCl_3$ solution containing ^{51}Cr in 11 ml of absolute ethanol and removing the H_2O by boiling until only 0.5 ml liquid remained. Next 134.2 mg of H(fod) in 1 ml of absolute ethanol was added and the mixture was heated for 1 hr at 80 °C. The solution was transferred to the vapor-phase separation apparatus, the ethanol was evaporated, and the chelate was sublimed at 140 °C. The 6.5-mg purple crystalline product was dissolved in 0.2 ml of dry Nanograde benzene for chromatographic injection.

Be(tfa)$_2$.-The radioactive metal chelate was prepared using a procedure similar to that of Berg and Truemper (312). A 41-mg portion of $BeSO_4 \cdot 4\ H_2O$ and 90.0 mg of NaAc were dissolved in 1.5 ml of H_2O. To this 0.25 ml of ^{7}Be solution and H(tfa) dissolved in 1 ml absolute ethanol were added. The Be(tfa)$_2$ was extracted using dry Nanograde benzene. The product was purified by vacuum sublimation at 65 °C. The pure radioactive chelate weighed 34.5 mg. It was dissolved in 1 ml of dry Nanograde benzene for chromatographic injection.

Fe(fod)$_3$.-The radioactive metal chelate was prepared by dissolving 1 mg of $FeCl_3$ in ethanol and adding ^{59}Fe solution and H(fod). The deep red chelate which formed was extracted into 2.5 ml of hexane. The hexane solution was placed in the improved vapor-phase separation apparatus, the hexane was evaporated, and the chelate was sublimed at 140 °C.

The 6-mg deep red product was dissolved in 0.4 ml of dry Nanograde benzene for chromatographic injection. The lower melting pale red portion was discarded.

Cu(tfa)$_2$.-The radioactive metal chelate was prepared using a procedure similar to that of Berg and Truemper (312). A 140.6 mg portion of $CuCl_2$ and 141.5-mg of NaAc were dissolved in 10 ml of H_2O. Next 305.5 mg of H(tfa) in 1 ml of ethanol was added. The insoluble product was extracted with dry Nanograde benzene and purified by vacuum sublimation at 115 °C. A 65.5-mg portion of the pure Cu(tfa)$_2$ was added to a mixture of 2 ml of dry Nanograde benzene and 65 mg of H(tfa). The solution was sealed in an ampoule and irradiated for 7.5 hr in the UFTR. The radiation damage to the chelate was annealed at 115 °C for 7 hr before opening the sealed irradiation ampoule. Next the chelate was purified by vacuum sublimation at 115 °C. A 54.4-mg yield of radioactive Cu(tfa)$_2$ was obtained. The pure chelate was dissolved in 1 ml of dry Nanograde benzene for chromatographic injection.

Gd(fod)$_3$.-The radioactive metal chelate was prepared by dissolving 89.3 mg of Gd(fod)$_3$ 87.5 mg of H(fod) in 3 ml of dry Nanograde benzene. One milliliter of this solution was irradiated in a sealed ampoule in port V-43 of the GTRR for 12.33 hr. The radiation damage to the chelate was annealed for 9.5 hr at 100 °C before opening the sealed irradiation ampoule. Vacuum sublimation at 150 °C yielded a yellow oil weighing 15 mg. This pure radioactive chelate was dissolved in 1 ml dry Nanograde benzene for chromatographic injection.

Lu(fod)$_3$.-The radioactive metal chelate was prepared by sealing 43.5 mg of Lu(fod)$_3$ and 42.1 mg of H(fod) in 1.5 ml of dry Nanograde benzene in an ampoule and irradiating in port V-43 of the GTRR for 14 hr. The radiation damage to the chelate was annealed at 100 °C for 10 hr before opening the sealed irradiation ampoule. A 33.5-mg yield was obtained from vacuum sublimation at 115 °C. The purified chelate was dissolved in 0.5 ml of dry Nanograde benzene for chromatographic injection.

Gas chromatography

Cr(tfa)$_3$.-The column, No. 1, Table 13, was conditioned overnight at 175 °C, treated with 10 µl of DMCS, and conditioned two more hours. Ten 5-µl injections of the radioactive Cr(tfa)$_3$ solution were made 1 min apart. The carrier flow and oven heat were kept on for 30 min after the last injection before dismantling the system for counting. The chelate solution was injected into three identical columns.

Cr(fod)$_3$.-The column, No. 1, Table 13, was conditioned overnight at 175 °C, treated with 10 µl of DMCS, and conditioned two more hours. Ten 5-µl injections of the radioactive Cr(fod)$_3$ solution were made 1 min apart. The carrier flow and oven heat were kept on for 30 min after the last injection before the system was dismantled for counting.

Be(tfa)$_2$.-The gas chromatographic conditions were taken from Ross and Sievers (181). After conditioning column No. 2, Table 13, for 30 min at 80 °C, twenty 5-µl injections of the radioactive Be(fta)$_2$ solution were made 1 min apart.

Table 13. Chromatographic Columns and Conditions

Column No.	Teflon Tube Dimensions	Liquid Phase	Solid Support	He Flow Rate, ml/min	Temperature, °C I.P.	Col.	Det.	Chelates
1	4 ft, 1/8 in	5% QF-1	80/100 mesh AW, DMCS Chrom. W	60	160 120 170	135 100 150	(150) (170)	$Cr(tfa)_3$ $Cr(hfa)_3$ $Cr(fod)_3$
2	4 ft, 1/8 in	5% SE-52	60/80 mesh Gas Chrom Z	50	170	80		$Be(tfa)_2$
3	7.5 ft, 1/8 in	10% SE-30	60/80 mesh Gas Chrom Z	30	205	170		$Fe(fod)_3$
4	2 ft, 1/8 in	5% SE-30	100/200 mesh Chrom. W	60	170	100		$Cu(tfa)_2$
5	6 in, 1/8 in	10% SE-30	80/100 mesh Chrom. W	30	230	170		$Lu(fod)_3$ $Gd(fod)_3$
6	2 ft, 1/8 in	20% High Vacuum Grease	100/120 mesh AW, DMCS Chrom. P	75	170	150		$Cu(hfa)_2$ $Cr(hfa)_3$
7	3 ft, 1/8 in	15% QF-1	80/100 mesh AW, DMCS Chrom. W	20	165	100		$Mn(hfa)_2$
8	5 ft, 1/8 in	20% High Vacuum Grease	100/120 mesh AW, DMCS Chrom. P	30	170	150	(170)	$Cu(hfa)_2$
9	1.5 ft, 1/8 in	20% SE-30	100/120 mesh AW, DMCS Chrom. P	30	150	150	(150)	$Cu(hfa)_2$

Table 13. continued

Column No.	Teflon Tube Dimensions	Liquid Phase	Solid Support	He Flow Rate, ml/min	Temperature, °C I.P.	Col.	Det.	Chelates
10	5 ft, 1/4 in	15% SE-30	80/100 mesh Chrom. W	100	170	150		$Cr(tfa)_3$

The carrier flow and oven heat were left on for 30 min after the last injection before dismantling the system for counting. Injections were made into three identical columns.

Fe(fod)$_3$.-The column, No. 3, Table 13, was conditioned at 200 °C for 15 min. Five 5-μl samples of the chelate solution were injected into the gas chromatograph 1 min apart. The carrier flow and oven heat were kept on for 45 min after the last injection before dismantling the system for γ-ray counting. Injections were made into three identical columns.

Cu(tfa)$_2$.-The gas chromatographic conditions were those used by Tanaka et al. (313). After conditioning column No. 4, Table 13, for 40 min at 100 °C, twenty 5-μl injections were made 1 min apart. After waiting 10 min after the last injection, the system was examined. The trap showed a well defined zone of blue chelate. Another less well defined blue region was seen through the Teflon tubing at the head of the column just beyond the heated injection port region. Presumably a cis-trans isomeric separation had taken place. The column oven was then reheated to 130 °C to elute the remaining chelate. After 10 min this was accomplished and after an additional 15 min the oven heat and carrier flow were cut off and the system dismantled for counting. The chelate solution was injected in the same manner into two more columns identical to the first, except operated exclusively at 130 °C.

Gd(fod)$_3$.-The experiment was done with three columns, No. 5, Table 13. One run was done with 10 injections of 5 μl

each. Two runs were done with 20 injections of 5 μl each. There was a 1-min interval between injections. The column oven heat and carrier flow were left on for 15 min after the last injection before dismantling the system for counting.

Lu(fod)$_3$.-The column, No. 5, Table 13, was conditioned for 1 hr at 170 °C. Ten 5-μl injections of the chelate solution were made 1 min apart. The oven heat and carrier flow were left on for 15 min after the last injection before dismantling the system for counting. Injections were made into three identical columns.

Cu(hfa)$_2$ and Cr(hfa)$_3$.-A 1-mg sample of each chelate was sealed in separate 2-mm o.d., 2-cm long quartz capillaries using a dry ice-acetone bath to cool the sample, thus preventing decomposition from the heat of the torch. The samples were irradiated for 4 hr in the UFTR.

The gas chromatographic column used for these injections was No. 6, Table 13. The sample was injected; the chelate eluted, was trapped, and the system was dismantled for counting.

Mn(hfa)$_2$.-A 1-mg solid sample of the chelate was sealed in a 2-mm o.d., 2-cm long quartz capillary and irradiated for 15 min in the UFTR. The sample was then injected as a solid into column No. 7, Table 13. After elution and trapping, the system was dismantled for counting.

Chelate residue exchange

An experiment was carried out to determine whether the incompletely eluted chelate residue which remained on the gas chromatographic column was inert to subsequently

injected samples of the same chelate, or whether there was exchange between samples injected at different times. A 1.09-mg solid sample of $Cu(hfa)_2$ was irradiated in the UFTR for 4 hr. It was then injected into column No. 8, Table 13 and eluted. Two hours later a similar solid sample of $Cu(hfa)_2$ containing no radioactive material was injected into the same column and the effluent was trapped and counted using the NaI(Tl) scintillation spectrometer to see if any ^{64}Cu from the first sample were present.

Silanization

An experiment was carried out to determine whether silanization of the injection port and column would significantly decrease metal chelate decomposition in the gas chromatograph or increase the amount of chelate detected in a given injection.

Two chelate solutions were prepared. One contained 29.0 mg of $Cr(fod)_3$ in 1 ml of dry Nanograde benzene; the other contained 29.0 mg of $Cr(tfa)_3$ in 1 ml of dry Nanograde benzene. Dimethyldichlorosilane (DMCS, PCR, Inc., Gainesville, FL) was used neat.

Two identical columns, No. 1, Table 13, were prepared. The column for the $Cr(fod)_3$ study was conditioned at 190 °C. The column for the $Cr(tfa)_3$ study was conditioned at 175 °C.

A series of 1-µl injections was made using a 1-µl syringe (Hamilton). Then a DMCS injection (10-40 µl) was made using a 100-µl syringe with a Teflon tipped plunger (Precision Sampling Corp., Baton Rouge, Louisiana). After

a short interval, typically 15 min, another series of 1-μl chelate injections and another DMCS injection were made. This was repeated three or four times until 70-80 μl of DMCS had been injected. A separate column was used for each of the chelates .

Counting geometry operation

Stainless steel spiral.-The operation of the geometry was evaluated using a chelate mixture consisting of 2.03 mg of $Cr(hfa)_3$ and 4.72 mg of $Cu(hfa)_2$. The mixture was sealed in a 2-mm o.d., 2-cm long quartz capillary and irradiated in the UFTR for 4 hr. The solid mixture was injected onto column No. 6, Table 13.

Elution was monitored using the NaI(Tl) scintillation spectrometer operating in the time sequenced store (TSS) mode. Each channel in the memory is assigned for storage sequentially; the number of input pulses which arrive during its assignment is stored. Thus a plot of gross counts versus elapsed time is obtained. The length of time each channel was active was 1.2 sec in this particular experiment. The solid sample injection and the beginning of TSS operation occurred at the same time.

Charcoal traps.-A fresh trap was attached before each component was eluted. After elution, the flow was stopped, the trap changed, and elution continued. The trap was then transferred to the counting equipment for measurement.

Simple reversible geometry.-The operation of the simple reversible geometry was evaluated by injecting a 2.26-mg solid

sample of $Cu(hfa)_2$ which has been irradiated in its injection capillary in the UFTR for 1 hr. The column used was No. 9, Table 13.

The chelate was injected into the gas chromatograph as the multichannel analyzer reached channel 45 in the TSS mode. The dwell time was 1.2 s per channel. The geometry temperature was 60 °C at injection, but was raised to 150 °C at channel 364 to release the chelate and purge the geometry.

Improved reversible geometry.-The operation of the improved reversible geometry was evaluated in a similar fashion. Twelve milligrams of $Cu(tfa)_2$ and 14 mg of H(tfa) were dissolved in 0.5 ml of benzene and irradiated in the UFTR for 7.5 hr. A 10-µl sample of this radioactive $Cr(tfa)_2$ solution was injected onto column No. 10, Table 13. The geometry inlet was operated at 150 °C, the geometry outlet was operated at 40 °C, and the CO_2 pressure was 20 psi. The multichannel analyzer was started in the TSS mode 6.5 min after sample injection. The dwell time was 1.2 s. After 45 min, the geometry purge was begun; the CO_2 flow was stopped, the geometry outlet heat was turned on, and another TSS mode measurement was begun.

Ion Exchange

A chelating ion exchange resin, Chelex-100 (Bio-Rad Laboratories, Richmond, California), was used in separation studies dealing with activation analysis of metal chelates in human serum. These studies included both preirradiation and postirradiation separations, along with both long and

short irradiation periods. The amount of water required for optimal sodium removal was also determined.

Postirradiation separation

Sodium separation optimization.-A 7.0-g serum sample was ashed and irradiated in the UFTR for 30 min. After irradiation, the ash was dissolved in 1 ml of 1M HNO_3 and the pH was adjusted to 7 with 15M NH_4OH.

The Chelex-100 was washed with deionized water and packed in a 0.8-cm i.d. Pyrex tube to a height of 6.0 cm. The column was eluted with 2 ml of 2M HNO_3 and washed with 60 ml of deionized water.

The radioactive ash solution was placed on the column along with 3 ml of deionized water and allowed to soak in for 5 min. Next the column was rinsed with 30 ml of deionized water at 0.5/min to remove the sodium. Each 5-ml portion of eluate was collected separately and its radioactivity, primarily due to ^{24}Na, was measured using the Ge(Li) detector and 400 channel analyzer.

Trace element determination

After sodium removal, the resin, which contained the trace elements, was placed in a counting vial and a γ-ray spectrum was recorded.

Preirradiation separation

Short irradiation.-The final location of the trace elements in the separation scheme was determined by analyzing the various system components: the resin before use, a solution blank, the eluate from the resin, and the resin used for the separation.

Resin blank.-The ion exchange column was prepared as described above and washed with 50 ml of deionized water, eluted with 50 ml of 2M HNO_3, and washed again with 50 ml of deionized water. At this point the column was ready for sample introduction. The top 80% of the resin was transferred to a quartz ampoule and sealed.

Solution blank.-The ion exchange column was prepared as described above. A blank was prepared by mixing 0.5 ml of 2M HNO_3, 0.5 ml of 2M KOH, and 2 drops of 15M NH_4OH and placing the mixture on the column. The mixture was allowed to soak in for 2 min. The column was then rinsed with 30 ml of deionized water and eluted with 30 ml of 2M HNO_3. All flow rates were 0.5 ml/min. The eluate was evaporated to 1 ml on a hotplate and sealed in a quartz ampoule.

Serum solution.-The ion exchange column used for the solution blank was rinsed with 50 ml of deionized water to prepare it for the serum ash solution. A 7.0-g serum sample was ashed in the low temperature dry asher for 12 hr at an rf power level of 200 W. The ash was dissolved in 1 ml of 2M HNO_3; the pH was adjusted to 7 with 2M KOH. A small amount of undissolved ash remained. The solution was decanted and placed on the column. The solids were dissolved, the solution was neutralized, and it was placed on the column along with 2 ml of deionized water. The mixture was allowed to soak in for 2 min. The column was then rinsed with 30 ml of deionized water and eluted with 25 ml of 2M HNO_3. The

flow rates were 0.5 ml/min. The eluate was evaporated to 1 ml on a hotplate and sealed in a quartz ampoule.

Finally, 80% of the ion exchange resin was removed from the column and sealed in a quartz ampoule.

Irradiation and counting.-All four ampoules were irradiated for 3 hr in the UFTR.

Their γ-ray spectra were recorded using the high-resolution γ-ray spectrometer system. Each of the four samples was counted within 4 hr of the irradiation.

Long irradiation.-The procedure was essentially the same as for the short irradiation, except that triplicate serum samples were included and no resin blank was processed.

The resin preparation was described above. Each serum sample weighed 6.2 g. The ash was dissolved in 0.8 ml of 2M HNO_3, and the pH was adjusted to 7 with 15M NH_4OH. The solution was placed on the Chelex-100 column and allowed to soak in for 2 min. The resin was rinsed with 25 ml of deionized water, eluted with 25 ml of 2M HNO_3, and the eluate was evaporated to 1 ml on a hotplate. The eluate, the blank, and 80% of the resin were then sealed in separate quartz ampoules. All five were irradiated in V-37 of the GTRR for 52.1 hr.

After irradiation, the samples were removed from the irradiation ampoules and placed in glass counting vials. The γ-ray spectra were recorded using the high resolution γ-ray spectrometer system described earlier.

CHAPTER III

RESULTS AND DISCUSSION

Objectives

The overall objective of this research is to expand the usefulness of NAA for trace analysis by developing new separation methods. These methods are in the areas of chelation, gas chromatography, and ion exchange. Work in these areas is unified by the goal of improved serum analysis, a powerful tool in medical and biochemical research.

The specific objective of the chelation studies is the demonstration of the principles involved in using chelates for trace element separation from matrices such as serum quantitatively, rapidly, reproducibly, and in a form suitable for further purification and resolution by gas chromatography. Efficient application of this technique to NAA requires the judicious choice of ligands, experimental conditions, and the proper sequence of operations, all of which are affected by both the elements of interest and the external demands on the analysis.

The objectives of the gas chromatographic studies are the demonstration and optimization of metal chelate separation as it applies to NAA. Goals of maximum resolution and counting efficiency, complete sample elution, ease of operation, and speed of analysis are sought through studying various methods

sample introduction and determining the elution characteristics of the chelates, with particular attention being paid to the extent to which each sample is eluted. Vapor and solid phase γ-ray counting devices must also be developed as an integral part of the gas chromatographic system to facilitate such analyses.

The specific objective of the ion exchange studies is the evaluation of chelating ion exchange resins as separation tools in trace element analysis in matrices such as serum. This includes optimizing conditions for sodium separation and evaluating subsequent trace element recovery.

Chelation Studies

The need for chemical separation in serum trace element analysis by NAA is apparent when γ-ray spectra recorded at various stages of an analysis are compared. Figure 5 from the first extraction experiment shows a 1-min γ-ray spectrum of untreated serum ash immediately after irradiation. The radiation from the ^{24}Na and ^{38}Cl obscures all other photopeaks. Figure 6 shows a 1-min γ-ray spectrum of the combined chelate solutions after direct chelation followed by chelation-extraction was performed on the irradiated ash. The interfering activity is greatly reduced, but ^{24}Na and ^{38}Cl still predominate. Figure 7 shows a 1-hr γ-ray spectrum of the volatile material trapped during vapor-phase separation of the chelate mixture. Some of the trace element photopeaks are now visible It is clear, then, that under these irradiation and decay conditions, chemical separation of some sort is needed.

Figure 5. Gamma-Ray Spectrum of Untreated Serum Ash.

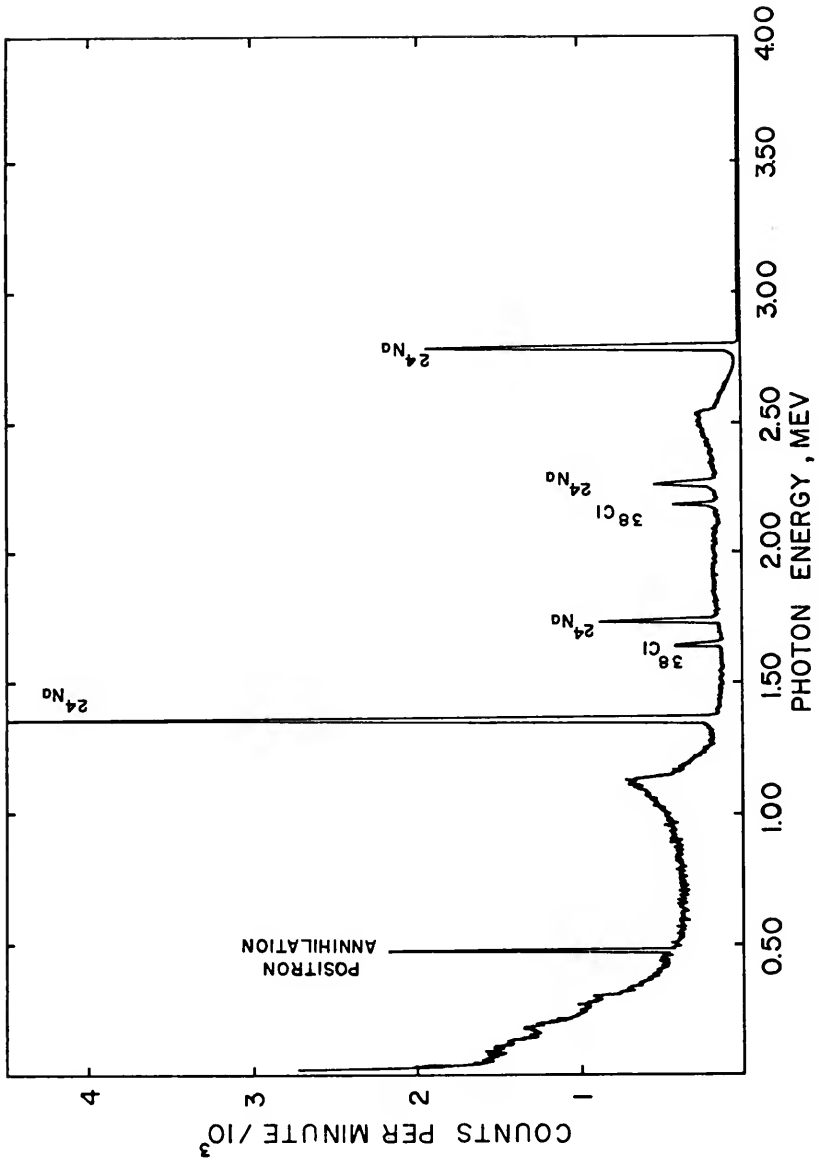

COUNTS PER MINUTE /10³
1
2
3
4
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
PHOTON ENERGY, MEV
POSITRON ANNIHILATION
24Na
38Cl
24Na
38Cl
24Na
24Na
24Na

Figure 6. Gamma Ray Spectrum of Chelate Solution After Extraction.

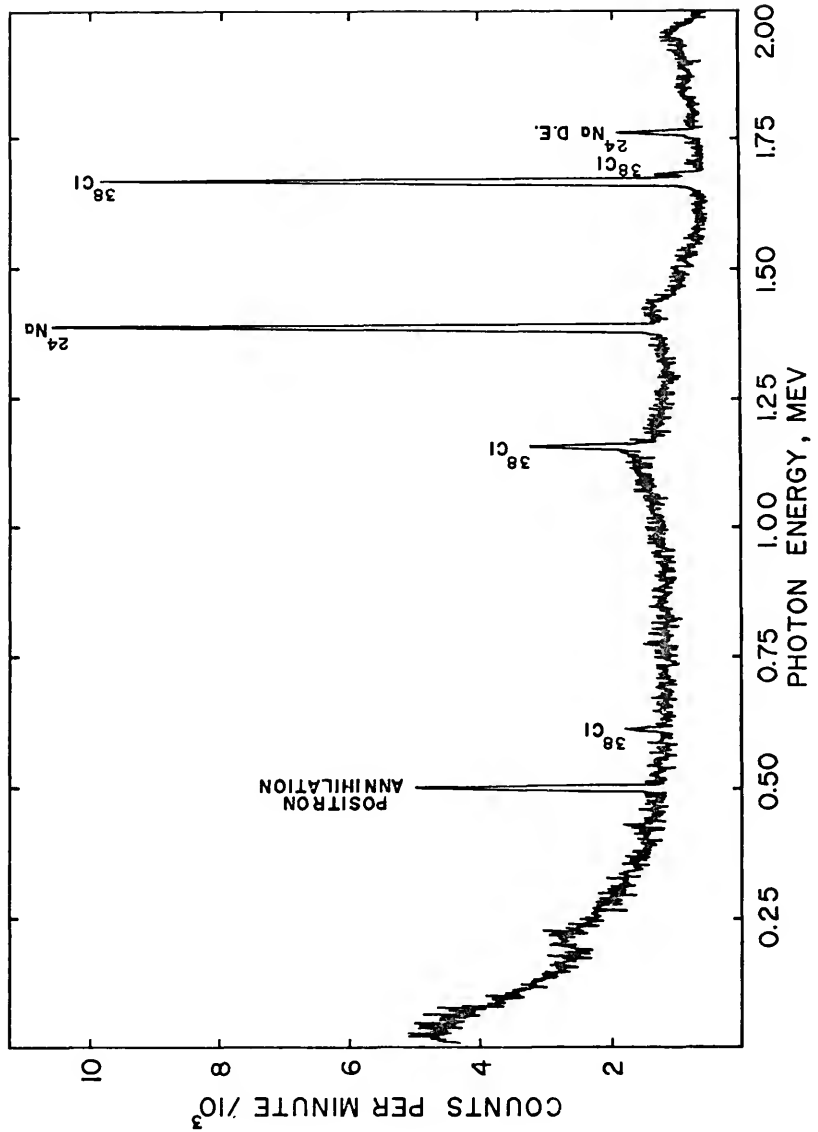
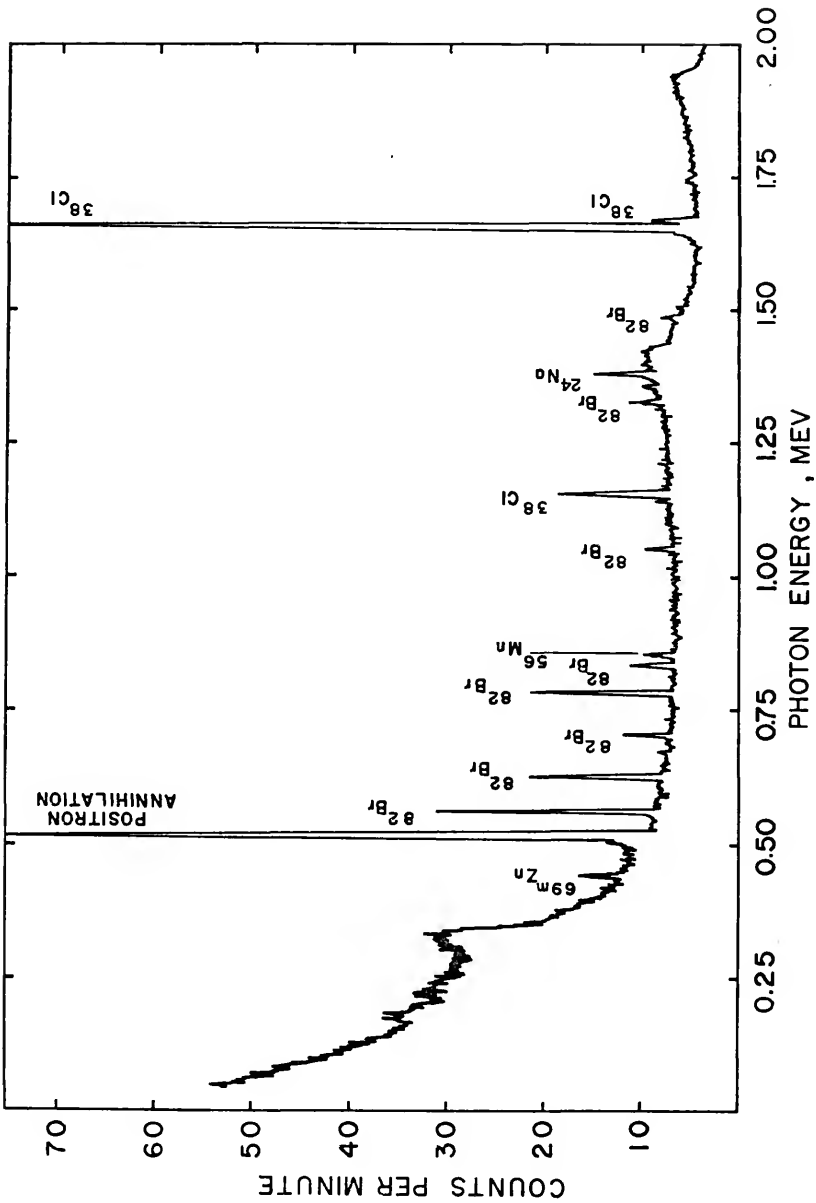

COUNTS PER MINUTE /10³
2
4
6
8
10
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
PHOTON ENERGY, MEV
POSITRON ANNIHILATION
38Cl
38Cl
24Na
38Cl
38Cl
24Na D.E.

Figure 7. Gamma-Ray Spectrum of Chelates After Vapor-Phase Separation.


COUNTS PER MINUTE
70
60
50
40
30
20
10
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
PHOTON ENERGY , MEV
^{38}Cl
^{82}Br
^{24}Na
^{56}Mn
^{69m}Zn
POSITRON ANNIHILATION

Chelation provides an effective means of separating metal ions which form stable chelates from other ions, such as sodium, which do not. The separation makes use of the change from ion to neutral complex during chelation. The low solubility of matrix ions in the organic solvent containing the chelates yields high separation factors. Many ligands have been used successfully for this type of separation, but ligands which form volatile metal chelates can undergo further purification in the vapor phase. This type of separation can be used with many elements; β-diketone chelates of about 60 metals have been reported (314). The volatility and thermal stability of the fluorine-substitited chelate allows even further sodium decontamination, since whatever sodium-containing material might be extracted will most likely not be sufficiently volatile to interfere in vapor-phase separation techniques. This is illustrated in Figures 5-7, where a sodium decontamination factor of about 1000 is shown. H(tfa) and H(fod) were chosen for reasons discussed in the Introduction: ease of chelate formation, lack of hydrolysis, excellent thermal stability, and when used together, ability to form complexes with a large number of elements.

Chelation Procedure

The low temperature dry asher used in these studies provided complete decomposition and oxidation of the dried serum samples in 12-14 hr with no apparent sample losses. Freeze drying was accomplished by placing frozen serum in the asher and immediately reducing the pressure to 1 Torr.

This eliminated foaming with accompaning sample loss, since only sublimation from the solid surface, not boiling, could take place. This evaporation combined with the low thermal conductivity of the low pressure atmosphere surrounding the serum to keep it frozen until all the water had been removed. A 6-ml serum sample yields about 40 mg of white, hygroscopic ash, a convenient size for processing.

This ash can be dissolved, the metal chelates being subsequently synthesized and isolated, or a chelation procedure using a direct reaction of the solid ash with the organic ligand solution can be used.

Chelation from solid ash

Advantages.-This direct approach, like the microreactor technique described by Sievers et al. for forming fod chelates directly from solid samples (192), has the advantages of being simple and rapid. In the preirradiation separations often required when long irradiations are used, additional sources of contamination are introduced by ash dissolution. In addition, since the extraction efficiencies of some metal chelates depend on pH to some extent, good pH control may be desirable. This is difficult in the small liquid volumes resulting from ash dissolution. Of course, since the ash is hygroscopic, care must be taken if all solvent extraction character is to be removed from the experiment.

Procedure.-The metal chelates were formed directly from the dry ash by mixing the ash with a benzene solution containing H(tfa) and H(fod). Since the bulk of the ash consisted of NaOH, no dissolution was apparent. The trace metals with

which the liquid came in contact did form soluble chelates. The trace elements were thus physically separated from the sodium in the ash when the mixture was centrifuged and the solution containing only trace metal chelates and ligand was decanted. At this point, the sodium concentration in the trace metal mixture had been lowered to a level which allowed trace metal determination by NAA without significant sodium interference.

Preirradiation separation.-Figure 8 shows the γ-ray spectrum of the benzene solution of the chelated trace metals after a 49-hr irradiation. Table 14 lists the radioisotopes found in this and two other spectra of the same mixture recorded after different decay periods. A total of 23 photopeaks representing 11 elements were identified. The later spectra show the complete decay of some iostopes. The decay of ^{82}Br and ^{24}Na allows identification of ^{124}Sb, ^{75}Se, ^{59}Fe, and ^{60}Co photopeak. More complete separation is thus needed where the delay required to detect these radioisotopes is unacceptable. The γ-ray spectrum from the solid residue recorded after a three week decay is shown in Figure 9. A total of 35 photopeaks representing 17 elements were identified. Table 15 contains a complete list of the radioisotopes which were detected in this and three other spectra of the same sample recorded after different decay periods.

In an attempt to chelate a larger number of metals, the experiment was repeated with additional mixing and more time for chelation. Figure 10 shows the γ-ray spectrum of the

Figure 8. Gamma-Ray Spectrum of Chelate Solution After Direct Chelation.

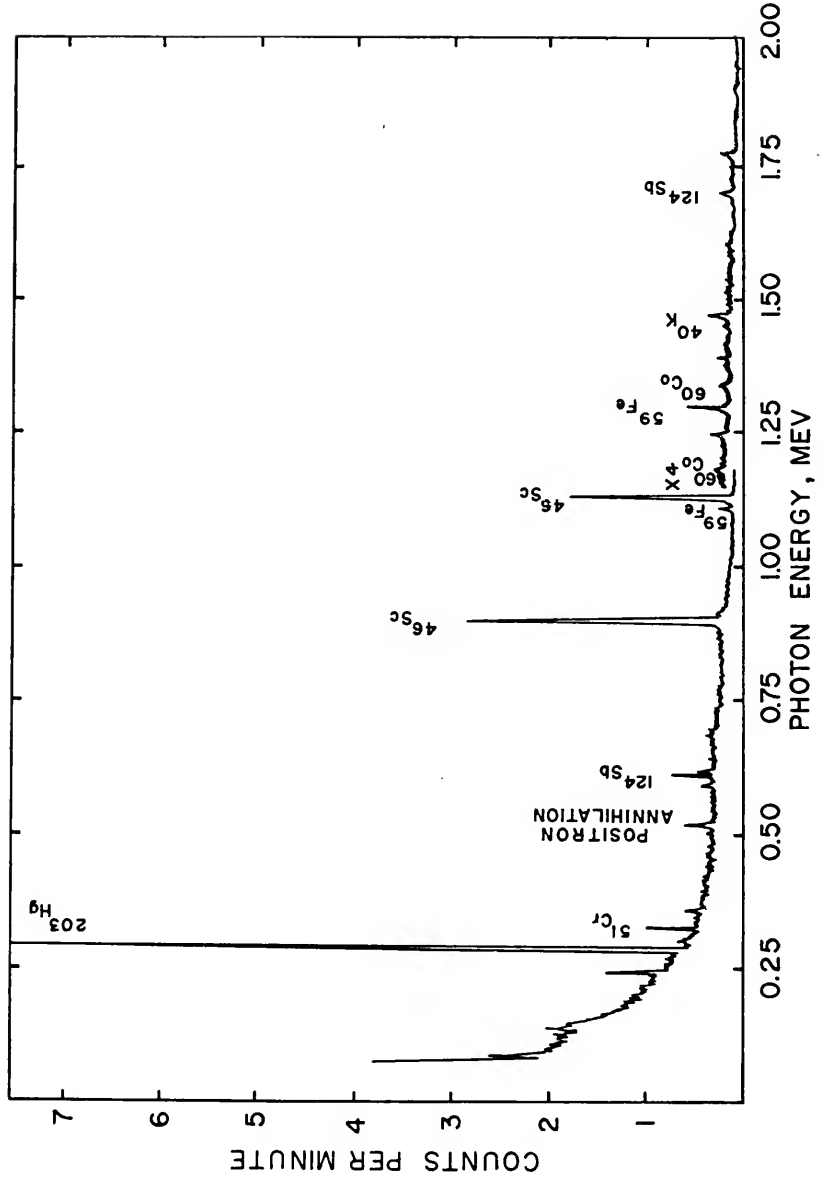

COUNTS PER MINUTE
PHOTON ENERGY, MEV
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
1
2
3
4
5
6
7
203Hg
51Cr
POSITRON ANNIHILATION
124Sb
46Sc
59Fe
46Sc
60Co x4
59Fe
60Co
40K
124Sb

Table 14. Spectra from Direct Chelation Organic Layer

Isotope	γ-Ray Energy, keV (315)	Spectra 7.5 day Decay	27 day Decay	37 day Decay
^{75}Se	136.0		X	X
^{82}Br	222	X		
^{75}Se	264.6		X	X
^{203}Hg	279.1	X	X	X
^{51}Cr	320.0	X	X	X
^{198}Au	411.8	X		
^{82}Br	554.3	X		
^{124}Sb	602.6		X	X
^{82}Br	619.0	X		
^{82}Br	698.3	X		
^{82}Br	776.6	X		
^{82}Br	827.8	X		
^{46}Sc	889.4	X	X	X
^{82}Br	1043.9	X		
^{59}Fe	1098.6		X	X
^{46}Sc	1120.3	X	X	X
^{60}Co	1173.1		X	X
^{59}Fe	1291.5		X	X
^{60}Co	1332.4		X	X
^{24}Na	1368.4	X		
^{82}Br	1474.7	X		
^{140}La	1595.4	X		
^{124}Sb	1690.7		X	X

Figure 9. Gamma-Ray Spectrum of Solid Residue AFter Direct Chelation.

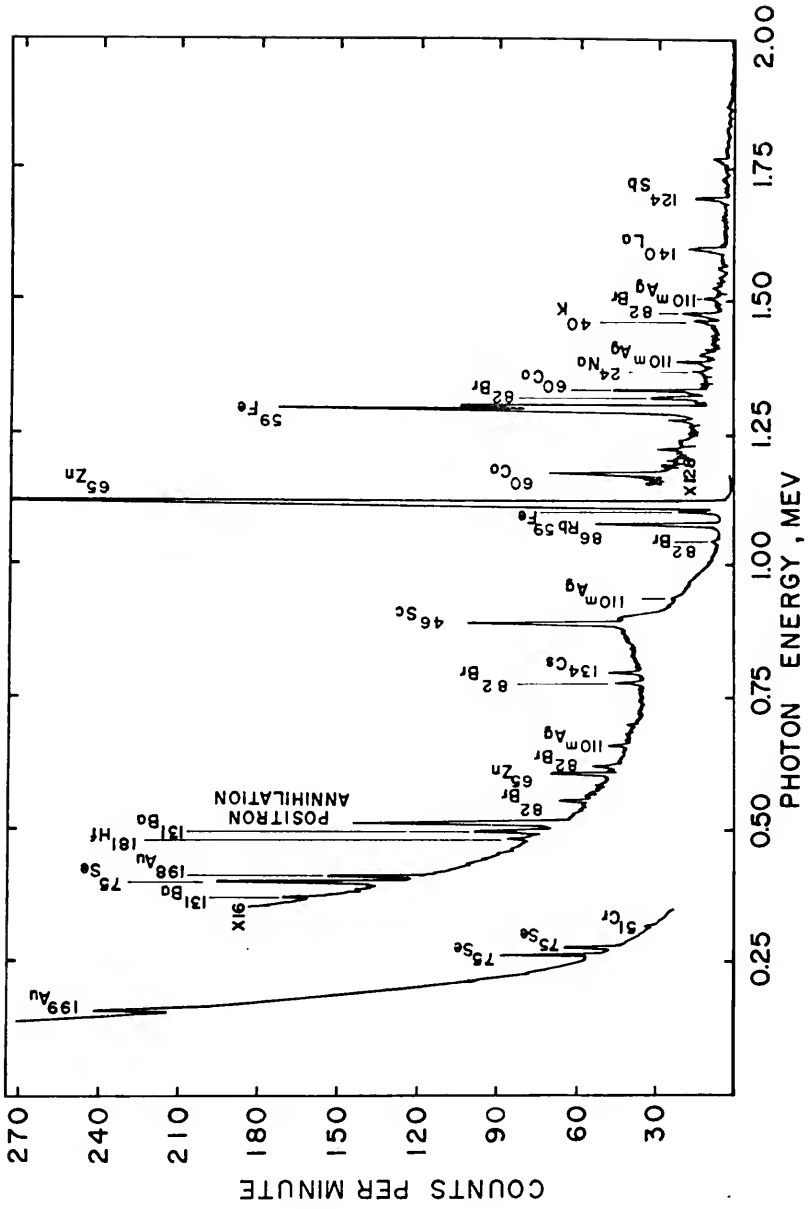

COUNTS PER MINUTE
270
240
210
180
150
120
90
60
30
PHOTON ENERGY, MEV
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
199Au
51Cr
75Se
75Se
91X
131Ba
198Au
75Se
181Hf
131Ba
POSITRON
ANNIHILATION
82Br
65Zn
82Br
110mAg
82Br
134Cs
82Br
46Sc
110mAg
82Br
86Rb
59Fe
65Zn
60Co
X28
59Fe
82Br
60Co
110mAg
24Na
40K
82Br
110mAg
140La
124Sb

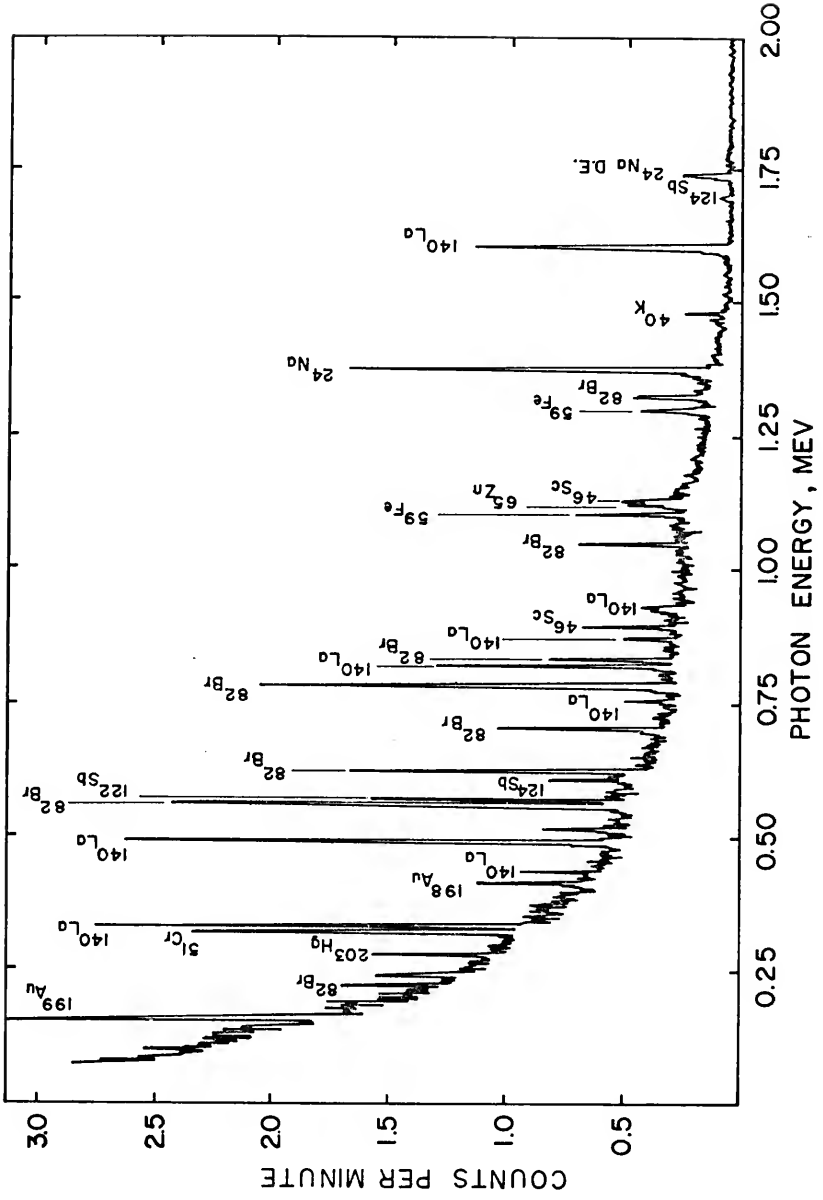
Table 15. Spectra from Direct Chelation Solid Residue

Isotope	γ-Ray Energy keV (315)	Spectra 21 day Decay	29 day Decay	46 day Decay	61 day Decay
^{75}Se	121.1	X	X	X	X
^{75}Se	136.0	X	X	X	X
^{199}Au	158.3	X	X		
^{75}Se	199				X
^{131}Ba	216.1	X	X		
^{75}Se	264.6	X	X	X	X
^{75}Se	279.6	X	X	X	X
^{75}Se	304				X
^{51}Cr	320.0	X	X	X	X
^{131}Ba	272.1	X	X		
^{198}Au	411.8	X	X		
^{75}Se	400.7	X	X	X	X
^{181}Hf	482.2				X
^{131}Ba	496.3	X	X	X	X
^{82}Br	554.3	X			
^{65}Zn	604.4	X	X	X	X
^{82}Br	619.0	X			
^{110m}Ag	657.8			X	X
^{82}Br	698.3	X			
^{82}Br	776.6	X			
^{134}Cs	795.8	X	X	X	X
^{46}Sc	889.4	X	X	X	X
^{110m}Ag	937.2	X	X	X	X

Table 15. continued...

Isotope	γ-Ray Energy keV (315)	Spectra 21 day Decay	29 day Decay	46 day Decay	61 day Decay
^{82}Br	1043.9	X			
^{86}Rb	1076.6	X	X	X	X
^{59}Fe	1098.6	X	X	X	X
^{65}Zn	1115.4	X	X	X	X
^{60}Co	1173.1	X	X	X	X
^{59}Fe	1291.5	X	X	X	X
^{82}Br	1317.2	X			
^{60}Co	1332.3	X	X	X	X
^{24}Na	1368.4	X			
^{110m}Ag	1384.0				X
^{82}Br	1474.7	X			
^{110m}Ag	1504	X	X	X	X
^{140}La	1595.4	X	X		
^{124}Sb	1690.1	X	X	X	X

Figure 10. Gamma-Ray Spectrum of Chelate Solution After Longer Direct Chelation.


COUNTS PER MINUTE
3.0
2.5
2.0
1.5
1.0
0.5
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
PHOTON ENERGY, MEV
199Au
82Br
203Hg
51Cr
140La
198Au
140La
140La
122Sb
82Br
124Sb
82Br
140La
82Br
140La
82Br
140La
46Sc
140La
82Br
59Fe
65Zn
46Sc
82Br
59Fe
24Na
40K
140La
124Sb 24Na D.E.

benzene solution of chelated trace elements thus obtained. A total of 32 photopeaks representing 13 elements can be identified. Table 16 contains a complete list of the isotopes which were found in this and two other spectra of the same material recorded after longer decay periods. The improved results after additional mixing were partly due to a shorter decay period, 4 days rather than 26 days, before counting. The total integrated flux was higher for the second sample. The enhanced sensitivity observed may have resulted to some extent from these factors rather than entirely from improved chelation conditions. Figure 11 shows the γ-ray spectrum of the solid residue after additional mixing. The 30 identified photopeaks from 16 radionuclides are listed in Table 17. They include all those identified in the preceding experiment and ^{181}Hf. The reagent blank spectrum photopeaks and radionuclides are listed in Table 18. Six elements present in the above spectra were found in the blank. Of these, however, only ^{92}Br, ^{203}Hg, ^{122}Sb, ^{124}Sb and ^{46}Sc originated entirely in the reagents used in the blank. The rest were also present in the serum.

Postirradiation separation.-An experiment involving a 30-min irradiation followed by direct chelation and a short γ-ray count revealed no trace metals. ^{128}I, ^{38}Cl, and ^{92}Br were the only radionuclides detected, other than small amounts of ^{24}Na contamination. One would expect that at least a few trace metals can be identified this way in spite of the short irradiation time. At this point, however, the data indicate

Table 16. Spectra from Longer Direct Chelation Organic Layer

Isotope	γ-Ray Energy keV (315)	Spectra 3 day Decay	5 day Decay	7 day Decay
^{82}Br	92	X		
^{199}Au	158.3	X	X	X
^{82}Br	222	X	X	X
^{203}Hg	279.1	X	X	X
^{51}Cr	320.0	X	X	X
^{140}La	328.6	X	X	X
^{24}Na	346.4	X		
^{198}Au	411.8	X	X	X
^{140}La	432	X	X	X
^{140}La	486.8	X	X	X
^{82}Br	554.3	X	X	X
^{122}Sb	564.0	X	X	X
^{124}Sb	602.6	X	X	X
^{82}Br	619.0	X	X	X
^{82}Br	698.3	X	X	X
^{140}La	751	X	X	X
^{82}Br	776.6	X	X	X
^{140}La	815.5	X	X	X
^{82}Br	827.8	X	X	X
^{24}Na	857.4	X		
^{140}La	867	X	X	X
^{46}Sc	889.4	X	X	X
^{140}La	925	X	X	X

Table 16. continued....

Isotope	γ-Ray Energy keV (315)	Spectra 3 day Decay	5 day Decay	7 day Decay
^{82}Br	1043.9	X	X	X
^{59}Fe	1098.6	X	X	X
^{65}Zn	1115.4			X
^{46}Sc	1120.3			X
^{59}Fe	1291.5	X	X	X
^{82}Br	1317.2	X	X	X
^{24}Na	1368.4	X	X	X
^{40}K	1460.7		X	X
^{82}Br	1474.7	X		
^{140}La	1595.4	X	X	X
^{124}Sb	1690.7	X	X	X
^{24}Na	1731.6	X	X	X

Figure 11. Gamma-Ray Spectrum of Solid Residue After Longer Direct Chelation.

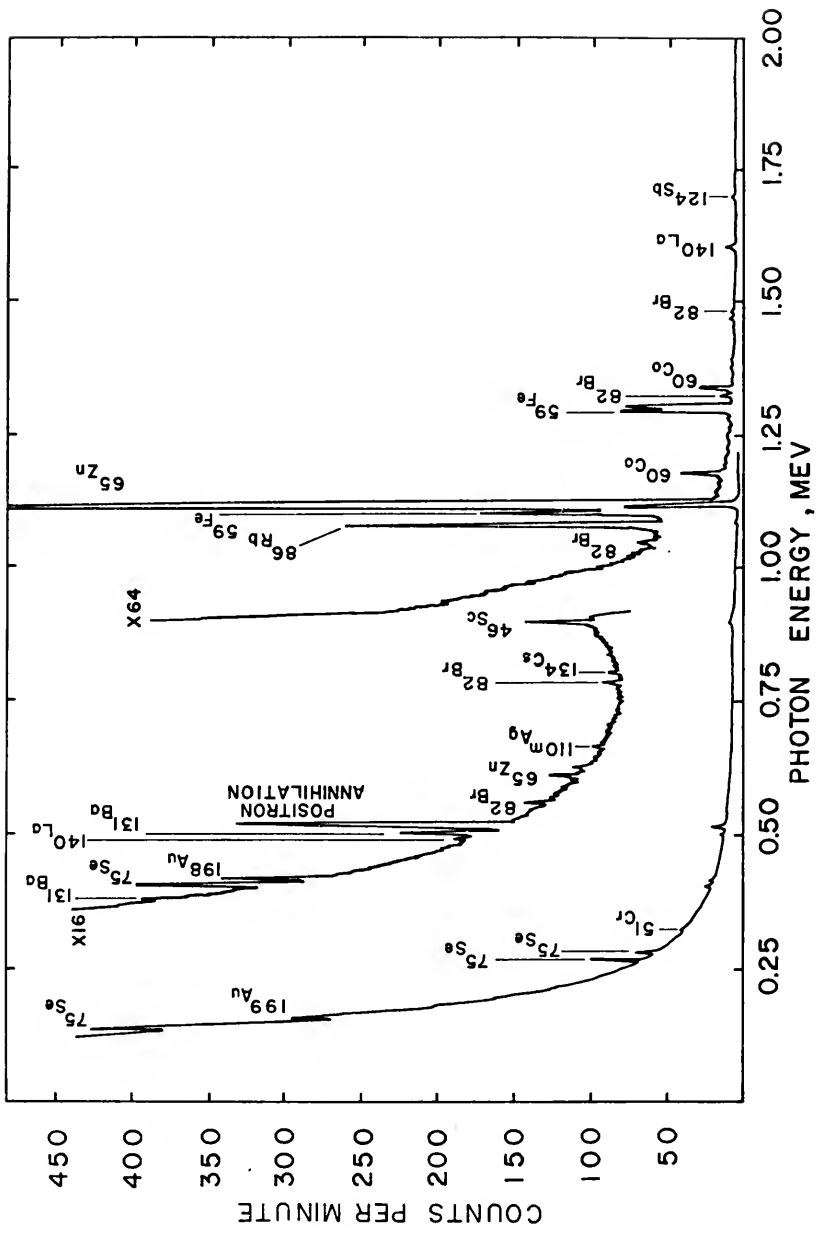

COUNTS PER MINUTE
450
400
350
300
250
200
150
100
50
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
PHOTON ENERGY, MEV
75Se
199Au
75Se
51Cr
75Se
198Au
131Ba
91X
131Ba
140La
POSITRON ANNIHILATION
82Br
65Zn
110mAg
134Cs
82Br
46Sc
X64
82Br
86Rb 59Fe
65Zn
60Co
59Fe
82Br
60Co
82Br
140La
124Sb

Table 17. Spectrum from Longer Direct Chelation Solid

Isotope	γ-Ray Energy keV (315)	Isotope	γ-Ray Energy keV
^{75}Se	121.1	^{82}Br	776.6
^{75}Se	136.0	^{134}Cs	795.8
^{199}Au	158.3	^{46}Sc	889.4
^{75}Se	264.6	^{82}Br	1043.9
^{75}Se	279.6	^{86}Rb	1076.6
^{51}Cr	320.0	^{59}Fe	1098.6
^{131}Ba	373.1	^{65}Zn	1115.4
^{75}Se	400.7	^{60}Co	1173.1
^{198}Au	411.8	^{59}Fe	1291.5
^{140}La	486.8	^{82}Br	1317.2
^{131}Ba	496.3	^{60}Co	1332.4
^{82}Br	554.3	^{24}Na	1368.4
^{65}Zn	604.4	^{82}Br	1474.7
^{82}Br	619.0	^{140}La	1595.4
^{110m}Ag	657.8	^{124}Sb	1690.1

Table 18. Spectra from Longer Direct Chelation Blank

Isotope	γ-Ray Energy keV (315)	4 day Decay	11 day Decay	15 day Decay
^{82}Br	92	X	X	
^{82}Br	222	X	X	
^{203}Hg	279.1	X	X	X
^{51}Cr	320.0	X	X	X
^{82}Br	274	X		
^{198}Au	411.8	X	X	
^{82}Br	554.3	X	X	
^{122}Sb	564.0	X	X	X
^{124}Sb	602.6	X	X	X
^{82}Br	619.0	X	X	
^{82}Br	698.3	X	X	
^{82}Br	776.6	X	X	
^{82}Br	827.8	X	X	
^{46}Sc	889.4	X	X	X
^{82}Br	952	X		
^{82}Br	1007	X		
^{82}Br	1043.9	X	X	
^{59}Fe	1098.6			X
^{46}Sc	1120.3	X	X	X
^{60}Co	1173.1	X	X	X
^{59}Fe	1291.5			X
^{82}Br	1317.2	X	X	
^{60}Co	1332.4	X	X	

Table 18. continued....

Isotope	γ-Ray Energy keV (315)	4 day Decay	11 day Decay	15 day Decay
^{24}Na	1368.4	X		
^{82}Br	1474.7	X	X	
^{82}Br	1650	X		
^{124}Sb	1690.1	X	X	X

that a preirradiation separation with its option of a long irradiation time offers the most promise for multielement determination.

Extraction from solution

Advantages.-The exposure of the atoms in the aqueous solution to the ligand is more uniform and more thorough, resulting in greater reproducibility and accuracy than can be obtained with the solid ash. Dissolving the ash allows metals which form oxides with differing stabilities to be uniformly available for chelation. Another advantage in dissolving the ash lies in being able to use a carrier solution. The carrier must be in the same chemical state as the sample. The difficulty of homogenous sampling of solid carrier along with the impracticality of duplicating the chemical state of the serum ash make solid carrier mixtures undesirable.

Prodedure.-The first extraction experiment used a procedure for determining a limited number of elements using a postirradiation separation with a simple carrier mixture. Spectra from this experiment appear in Figures 5-7. Ash dissolution and chelate extraction were preceded by a direct chelation step. Any metals which are preferentially chelated this way are removed under optimum conditions, while the others are unaffected and remain with the ash to be chelated after dissolution.

The results of a procedure for determining a large number of elements using a postirradiation separation with a complex

carrier mixture appear in Table 19. It lists photopeaks present in the γ-ray spectra recorded at various stages of each of three experiments using the multielement carrier solution. The items whose activities were measured are listed in the source column. The designation total organic refers to the combined organic extract solutions. Counting took place after chelate extraction but before vaporization. The traps were charcoal filled; their numbers indicate the sequence in which they were attached to the outlet of the vaporization system. The pot contained the nonvolatile residues after vaporization was complete. The time and temperature column entries are the length of time each trap was attached at the specified vaporization temperature. The other two columns contain the major and minor radionuclides observed. The underlined components make up an unusually large proportion of the total activity present.

Adjustment of pH in the ash solution after acid dissolution is important for several reasons. As mentioned in the introduction, the pH should be between 5 and 8 for maximum extraction efficiency using the ligands H(tfa) and H(fod). Perhaps more important than the accuracy of the adjustment are the complexing characteristics of the base used for neutralization. In experiments B and C, KOH was used for neutralization. A substantial amount of precipitate was formed in both cases. The precipitated metals are closer to being completely available for chelation than were their oxides in the ash, yet chelate formation still cannot take place

Table 19. Direct Extraction Complex Carrier Experiment

Source Exp. A	Time and Temperature	Major Photopeaks	Minor Photopeaks
Total Organic		^{24}Na ^{82}Br	
Traps 1-5		^{82}Br ^{64}Cu	^{38}Cl ^{24}Na ^{128}I
Traps 1 & 2	7 min, 100 °C	^{82}Br	^{28}Cl ^{64}Cu
Trap 3	5 min, 150 °C	^{64}Cu	^{82}Br
Trap 4	5 min, 175 °C	none	^{82}Br
Trap 5	10 min, 200 °C	none	^{24}Na
Exp. B			
Total Organic		^{38}Cl ^{128}I	^{24}Na ^{82}Br
Traps 1-5		^{24}Na ^{82}Br	^{28}Cl ^{128}I
Traps 1 & 2	3 min, 100 °C 3 min, 140 °C	^{82}Br	^{24}Na ^{38}Cl ^{64}Cu
Trap 3	3 min, 170 °C	^{24}Na	^{82}Br ^{64}Cu
Trap 4	4 min, 200 °C	^{24}Na	none
Trap 5	4 min, 200 °C	none	none
Pot		^{82}Br ^{24}Na	^{64}Cu

Table 19. continued....

Source Exp. C	Time and Temperature	Major Photopeaks	Minor Photopeaks
Total Organic		^{24}Na ^{38}Cl	none
Traps 1-4		^{82}Br	^{24}Na ^{38}Cl ^{64}Cu ^{128}I
Precipitate		^{24}Na ^{56}Mn	^{139}Ba ^{87m}Sr ^{82}Br
Trap 1	3 min, 100 °C	^{82}Br	^{24}Na ^{38}Cl
Trap 2	3 min, 140 °C	^{24}Na	^{64}Cu
Trap 3	3 min, 170 °C	none	^{24}Na
Trap 4	15 min, 200 °C	none	^{24}Na
Traps 1-4		^{82}Br ^{24}Na	^{64}Cu

directly. The same thing is true when NH_4OH is used for neutralization. Here, some of the metals are present as complexes. Assuming the pH is kept low enough to prevent sequestration, these complexes must be capable of rapid dissociation to allow rapid formation of the metal chelates. In such a solution, where none of the molecules can be completely surrounded by less-reactive species and thus kept from reacting, one would expect better results. The presence of ^{56}Mn, ^{139}Ba and ^{87m}Sr in the precipitate in experiment C, even after repeated washing, tends to support this conclusion. β-diketone chelates of all three are known, yet even at a suitable pH they remained as hydroxide precipitates.

Carrier Preparation

The use of a carrier is sometimes necessary when unfavorable equilibria combine with low concentrations to make efficient recovery of radioisotopes in postirradiation separation procedures difficult. If only a few elements are sought, carrier preparation may consist of simply adding small amounts of salts of the elements of interest to the serum ash just before dissolving it. Results of such an experiment appear in Figures 5-7. Photopeaks from ^{64}Cu, ^{56}Mn, and ^{69m}Zn appear in Figure 7, the spectrum from the separated chelates. Their presence shows that a carrier solution is neither necessary nor sufficient for the detection of a particular element in every case since no Zn or Mn carrier was present, yet Cr, La, and Sm carrier addition did not result in detection of these elements.

The use of a complex carrier solution in a survey-type NAA experiment requires choosing the carrier elements carefully and systematically. Care must be taken to include every isotope which might be detected under the unique set of irradiation and counting conditions encountered in a particular experiment. Since the iostopes produced by (n, γ) reactions have a variety of half-lives as well as different parent cross sections and abundances, it is not always immediately obvious whether or not a particular element will yield significant activity after irradiation under a particular set of conditions. Equation 1 can be used to calcualte this activity.

$$A = N\sigma\phi \quad (1-e^{-\gamma t_i}) \quad (e^{-\gamma t}) \qquad (1)$$

where A = activity in disintegrations per second per microgram

N = number of target atoms

σ = thermal neutron cross section

ϕ = thermal neutron flux

γ = decay constant

t_i = irradiation time

t = decay time

The use of a computer facilitates calculations for many elements under different sets of conditions. A Focal program used with the PDP-8/L computer for these calculations appears in Table 20. This particular program calculates the activity per microgram of parent after a 30 min irradiation at 10^{12}n cm^{-2} s^{-1} and a 1 hr decay. The results of the calculations for 105 isotopes appear in Table 21.

Table 20. Activity Calculation Program

```
    C-FOCAL8-UF 8K3W

01.01 C PROGRAM TO CALCULATE ACTIVITY IN DISINTEGRATIONS
01.02 C PER SECOND PER MICROGRAM AFTER A 30 MINUTE
01.03 C IRRADIATION AT A FLUX OF 10^12 AND A 1 HOUR
01.04 C DECAY.

01.15 E
01.20 S L2 = -FLOG(2)
01.25 T "                                                PARENT"
01.26 T "     PARENT "!
01.30 T "                          DPS/UG      HALF    %ABUN-"
01.31 T "       CROSS    ATOMIC"!
01.40 T "            ISOTOPE  OF PARENT    LIFE     DANCE"
01.41 T "    SECTION    WEIGHT"!!!
01.60 T "              ";X =;X :;A W;X E;C REMOVE ECHO.
01.65 T #"                                    "
01.70 A HL;C HALF LIFE IN HOURS.
01.75 T %6.02 HL;T " ";A AB;T %5.02 AB;T " "
01.80 A XS;C CROSS SECTION IN BARNS
01.83 T %8.03 XS;T "  "
01.85 A WT;T %4.01 WT
01.90 T #"                          "
02.10 S P1=6.023E05*XS*<1-FEXP(L2/(2*HL))>
02.20 S P2=FEXP(L2/HL)*AB/(100*WT)
02.30 S N=P1*P2;
03.05 S X=0
03.10 I (N-1)3.3;I (10-N)3.5,3.5
03.20 T %3.02 N;T "E";T %1 X;T !
03.22 X R;C RESTORE ECHO, "=", AND ":"
03.25 GOTO 1.6
03.30 S N=N*10;S X=X-1;G 3.1
03.50 S N=N/10;S X=X+1;G 3.1
```

Table 21. Activity Calculations

ISOTOPE	DPS/UG OF PARENT	HALF LIFE	PARENT %ABUN-DANCE	PARENT CROSS SECTION	ATOMIC WEIGHT
NA24	2.28E 2	15.00	100.00	0.400	23.0
MG27	9.66E-1	0.16	11.17	0.030	24.3
SI31	6.30E 0	2.62	3.09	0.100	28.1
P32	3.72E 0	343.00	100.00	0.190	31.0
S35	2.55E-2	2150.00	4.22	0.200	32.1
CL38	2.50E 2	0.62	24.47	0.430	35.5
AR37	1.27E-1	840.00	0.34	6.000	39.9
AR41	1.15E 3	1.83	99.60	0.650	39.9
K42	3.59E 1	12.40	6.88	1.300	39.1
CA45	2.98E-2	3960.00	2.06	1.100	40.1
CA49	2.64E-1	0.15	0.18	1.100	40.1
SC46	5.77E 1	2010.00	100.00	25.000	45.0
V52	5.43E-1	0.06	99.76	4.800	50.9
CR51	4.15E 0	666.00	4.31	16.000	52.0
MN56	1.40E 4	2.58	100.00	13.300	54.9
FE59	1.40E-2	1083.00	0.33	1.230	55.8
CO60	1.28E 0	46100.0	100.00	17.000	58.9
NI65	1.64E 1	2.52	1.08	1.520	58.7
CU64	7.40E 2	12.90	69.09	4.500	63.5
ZN65	2.18E-1	5850.00	48.89	0.820	65.4
ZN69M	3.61E 0	13.90	18.57	0.090	65.4
ZN69	2.52E 2	0.97	18.57	1.000	65.4
ZN71M	3.59E-1	4.00	0.62	0.090	65.4
GA72	1.19E 1	14.10	39.60	0.150	69.7
GE75	5.70E 1	1.38	36.54	0.140	72.6
AS76	4.38E 2	26.50	100.00	4.300	74.9
SE75	4.37E-1	2890.00	0.87	55.000	79.0
SE81M	4.48E 1	0.95	49.82	0.080	79.0
BR82	9.35E 0	35.50	49.46	0.260	78.9
KR79	4.87E-1	34.90	0.35	2.000	83.8
KR85M	2.65E 1	4.39	56.90	0.100	83.8
KR87	1.04E 1	1.26	17.37	0.060	83.8
RB88	1.57E 1	0.30	27.85	0.120	85.5
SR85M	3.12E 0	1.16	0.56	0.570	87.6
SR87M	4.89E 1	2.83	9.86	0.800	87.6
SR89	7.87E-3	1248.00	82.56	0.005	87.6
Y90M	5.73E-1	3.10	100.00	0.001	88.9
ZR95	1.91E-2	1560.00	17.40	0.075	91.2
NB94M	9.20E-1	0.10	100.00	0.150	92.9
MO93M	2.64E-1	6.90	15.84	0.006	95.9
MO99	1.07E 0	66.69	23.78	0.140	95.9
MO101	5.30E 0	0.24	9.63	0.200	95.9
RU97	3.39E-1	69.60	5.51	0.210	101.1
RU103	9.61E-1	950.00	31.61	1.400	101.1
RU105	3.34E 1	4.44	18.58	0.470	101.1

Table 21. continued...

ISOTOPE	DPS/UG OF PARENT	HALF LIFE	PARENT %ABUN-DANCE	PARENT CROSS SECTION	ATOMIC WEIGHT
PD109	4.38E 2	13.47	26.71	12.000	106.4
PD111	2.46E 1	0.37	11.81	0.400	106.4
AG110M	6.41E-1	6090.00	48.18	4.200	107.9
CD107	3.05E 0	6.50	1.22	1.000	112.4
CD111M	9.83E 0	0.82	12.39	0.100	112.4
CD115M	1.87E-1	1032.00	28.86	0.360	112.4
CD116	8.67E-1	3.40	7.58	0.027	112.4
IN114M1	2.92E-1	1200.00	4.28	4.500	114.8
IN116M1	5.35E 4	0.90	95.72	72.000	114.8
SN113M	2.45E 0	0.67	0.96	0.350	118.7
SN117M	4.48E-3	336.00	14.30	0.006	118.7
SN121	7.58E-1	27.00	8.58	0.140	118.7
SN125M	4.91E-1	0.16	5.94	0.140	118.7
SB122	8.92E 1	67.30	57.25	6.200	121.8
SB124M2	2.75E 0	0.35	42.75	0.015	121.8
SB124	1.73E 0	1440.00	42.75	3.400	121.8
TE127	2.67E 1	9.40	18.71	0.900	127.6
TE131M	3.65E-1	30.00	34.48	0.020	127.6
TE131	3.49E 1	0.42	34.48	0.200	127.6
I128	3.17E 3	0.42	100.00	6.200	126.9
XE133M	4.11E 0	54.30	26.89	0.530	131.3
XE135M	5.64E 0	0.26	10.44	0.230	131.3
CS134M	1.05E 3	2.90	100.00	2.600	132.9
BA139	1.48E 2	1.38	71.66	0.350	137.3
LA140	3.22E 2	40.20	99.91	8.800	138.9
CE137M	7.62E-2	34.40	0.19	0.950	140.1
CE137	1.80E 0	9.00	0.19	6.300	140.1
CE143	4.63E 0	33.00	11.07	0.950	140.1
PR142	2.88E 2	19.20	100.00	3.900	140.9
ND147	1.34E 0	266.00	17.62	1.400	144.2
ND149	7.28E 1	1.73	5.73	2.500	144.2
SM153	4.69E 3	46.80	76.72	210.000	150.4
SM155	4.67E 2	0.37	22.71	5.500	150.4
EU152M2	9.09E 2	1.60	47.82	3.800	152.0
EU152M1	1.95E 5	9.20	47.82	3000.000	152.0
GD159	6.12E 1	18.00	24.87	3.500	157.3
TB160	2.25E 1	1750.00	100.00	30.000	158.9
DY157	2.22E-1	8.10	0.05	3.000	162.5
HO166	2.79E 3	26.90	100.00	61.200	164.9
ER163	1.12E 2	1.25	0.14	160.000	167.3
ER171	1.32E 2	7.52	14.88	6.000	167.3
YB175	2.45E 2	101.00	31.84	65.000	173.0
LU176M	5.22E 3	3.70	97.41	21.000	175.0
HF180M	8.49E 0	5.50	13.75	0.340	178.5
HF181	5.09E 0	1020.00	35.24	12.600	178.5

Table 21. continued...

ISOTOPE	DPS/UG OF PARENT	HALF LIFE	PARENT %ABUN-DANCE	PARENT CROSS SECTION	ATOMIC WEIGHT
TA182M	1.92E 0	0.27	100.00	0.010	180.9
W187	4.80E 2	24.00	28.41	37.000	183.8
RE186	5.03E 2	90.00	37.07	110.000	186.2
RE188M	1.92E 2	0.31	62.93	1.300	186.2
RE188	2.61E 3	12.70	62.93	70.000	186.2
OS190M	6.93E-3	0.17	16.10	0.001	184.0
OS191M	2.59E 2	13.00	26.40	12.000	184.0
OS191	3.32E 0	360.00	26.40	4.000	184.0
IR194	4.10E 3	17.40	62.70	110.000	192.2
PT195M	3.08E-1	98.50	32.90	0.087	195.1
PT197M	6.38E 0	1.33	25.30	0.060	195.1
AU198	1.60E 3	64.60	100.00	98.800	197.0
HG199M	8.77E-1	0.72	10.02	0.020	200.6
HG203	1.36E 0	1118.00	29.80	4.900	200.6
PB209	2.50E 0	3.30	52.30	0.020	204.0

These data were used to choose the elements listed in Table 12. Various salts of these elements were dissolved to make a carrier solution. Concentrations of 0.01M were chosen so as to minimize the volume of carrier solution added to each ash sample and thus avoid unnecessary ash solution dilution. However, overnight clouding of the carrier solution and the appearance of metallic gold particles from oxidation-reduction reactions were noted. Undoubtedly this resulted in some of the elements in the carrier solution being in a non-ionic form and therefore not available for chelation. These reactions could have even continued in the serum ash solution after carrier addition. Lower concentrations, along with either use of the carrier solution immediately after preparation or the segregation of the more reactive ions into separate solutions, would probably eliminate this and result in a more homogeneous mixture.

Additional Separation

Further separation of the fluorine-substituted β-diketonates by vaporization and gas chromatography is possible because of the chelates' thermal stability and volatility. The first extraction experiment uses the first of two vapor-phase separation apparatus designs which were constructed using the oven and carrier gas flow system from a gas chromatograph. The chelates passed easily from the injection port to the charcoal trap, while a significant amount of white crystalline residue remained in the injection port liner and the first inch of the 6-in long open Teflon tube. So even

though the previous solvent extraction step separated the chelate mixture from material which was insoluble in benzene, the useful data in Figure 7 was obtained only after the non-volatile material had been removed also. The spectra of this white residue left behind show only ^{24}Na.

The second vapor-phase separation apparatus is a higher capacity version of the first. A liquid reservoir with milliliter capacity acting as a distillation pot in the oven eliminated tedious serial syringe injections into the injection port. Furthermore, uniformly increasing heat applied simultaneously to the entire sample allows chelate vaporization in order of decreasing volatility after solvent removal is complete. This prevents the higher temperatures required for vaporizing certain chelates from causing decomposition of more sensitive, more volatile chelates which can be eluted intact at lower temperatures. Also, solvent condensate in the trap cannot wash condensed chelate out of the trap when solvent removal takes place first.

The effectiveness and versatility of the second vapor-phase separation apparatus is illustrated by the separation data in Table 19. Experiment A shows that the activated metals, ^{64}Cu in this case, come out in relatively narrow bands rather than being spread out over the entire 27-min heating period. Trap 5, experiment B, shows that the separation is rapid and efficient, as no more radioactive material is eluted after 4 min of high temperature operation. The material of interest in A eluted after ^{82}Br and ^{38}Cl, but

before ^{24}Na. Resolution is also apparent in experiment B, although some ^{82}Br, ^{38}Cl, and ^{24}Na contaminated the ^{64}Cu spectra. Here, the temperature was raised to the Cu-elution level before all the Cl and volatile Br had been eluted. Interference from ^{24}Na is also present in trap 3 because of too rapid heating.

The presence of ^{64}Cu and a significant amount of ^{82}Br in the distillation pot after 8 min at 200 °C in experiment B indicates the presence of both radioisotopes in nonvolatile compounds. Since no attempt was made to influence the Br reactions in the preceding steps it is not surprising that Br compounds with widely varying volatilities were formed. Every attempt was made to chelate quantitatively the Cu, however. The Cu left in the pot is probably the result of decomposition during vaporization. Gentler heating should help reduce this loss.

There is another way of obtaining volatile chelate fraction resolution which can be used equally well with either of the vapor-phase separation apparatus designs. If the carrier gas flow, temperature gradient, and diameter of the Pyrex trap tube used for trapping the chelates are properly chosen, the components of the mixture will condense in separate, distinct bands as they encounter successively cooler regions farther from the oven. This procedure does not offer high resolution, but its effectiveness is sufficient for many separation situations.

Interferent Removal

Whether the serum spectrum is dominated by the short half-life radioisotopes such as 25.4-min ^{128}I and 37.29-min ^{38}Cl, or the longer half-life radioisotopes such as 15-hr ^{24}Na and 35.82-hr ^{82}Br, depends on the length of the irradiation and how soon afterward chelation can be performed and the γ-ray spectrum can be recorded. The preirradiation direct chelation procedure yielded a chelate mixture whose γ-ray spectrum had no ^{24}Na interference after a 3-day decay. The chelate-containing organic layer spectrum summarized in Tables 14 and 16 contain photopeaks from ^{198}Au, ^{82}Br, and ^{140}La, all of which would have decayed undetected had the sodium not been removed.

The spectrum of the chelate extract solution from the postirradiation chelate extraction procedure, Figure 10, contained significant ^{24}Na interference. Vapor-phase separation of this solution reduced the ^{24}Na by a factor of at least 500, leaving ^{38}Cl as the major interferent. At this point, previously obscured ^{64}Cu, ^{69m}Zn and ^{56}Mn photopeaks can be detected. The Na decontamination factors for both these procedures are between 10^3 and 10^4.

The experimental results from the solution extraction with a complex carrier mixture (Table 19) were not completely without ^{24}Na interference even after a final distillation. As was expected, all three organic extracts contained significant ^{24}Na contamination, but experiments B and C had additional contamination in the traps after distillation. The

mechanism by which the ^{24}Na reached the traps is not known with certainty. Perhaps a volatile sodium-containing compound was formed. What is more likely is that solid particles were blown from the distillation pot into the traps. The use of glass wool plugs in the system may correct this. It must be remembered that the ^{24}Na seen in these traps represents an extremely small fraction of the ^{24}Na present before separation. Another useful observation is that, rather than flowing continuously into the traps at every temperature, the bulk of the flow took place at higher temperatures. This fact permits further ^{24}Na separation from the more volatile chelates where necessary.

Once the ^{24}Na has been removed, the halogen interference, particularly from the ^{82}Br with its numerous photopeaks, is the major problem. Serum contains a large amount of Cl and lesser amounts of Br and I. During activation these atoms are made highly reactive by Szilard-Chalmers reactions. Their chemical state after irradiation and chelate formation is undoubtedly significantly altered and probably diverse and complex. Collman has reported direct substitution of halogens into β-diketone chelate rings (316). Although the number of chelate molecules thus affected is insignificant, the presence of ^{82}Br in a form with properties similar to the chelates of interest could make interference removal somewhat more complicated.

Since the halogens and their compounds are often volatile, the separation techniques described here are not nearly as

effective for them as for sodium. The ^{82}Br interference problem in the preirradiation direct chelation experiments is shown in Figure 7. Of course it is of nowhere near the same magnitude as the previously illustrated ^{24}Na interference. Nevertheless, its removal would facilitate trace element photopeak measurement.

The postirradiation extraction experiments, with their short irradiation and decay periods, are particularly vulnerable to interference from 37.39 min ^{38}Cl. Figure 9 shows the prominence of this radioisotope's photopeaks before separation. When Figures 9 and 10 are compared, it can be seen that the separation was much more effective for Na than Cl. This is apparent since the relative peak heights are more nearly the same in the later spectrum in spite of the fact that the time which elapsed between them represented several ^{38}Cl half-lives but less than one ^{24}Na half-life. Further decay and separation yield nearly complete ^{24}Na removal, but the ^{38}Cl, even after undergoing a decay of several half-lives, interferes significantly.

Further separation of the volatile components is the solution to these halogen interference problems. The three complex carrier experiments show how this separation can be accomplished. For example, experiment A shows that ^{38}Cl interference with ^{64}Cu measurement can be completely eliminated by changing traps after 6 min at 100 °C. Another method is used with the other system. The temperature remains constant; the separated fractions appear as distinct bands in the trap.

Annealing Studies

Annealing recoil damage in irradiated metal chelates is necessary when both chelation before irradiation and further chelate separation after irradiation are required. Chelation before irradiation provides a simple means of avoiding the hazardous ^{24}Na radiation levels encountered when irradiating beyond 30 min. Further chelate separation must be delayed until after irradiation if the separated fractions are to be counted separately. This postirradiation separation allows simultaneous irradiation of the entire sample, reduces irradiation container handling, and makes the use of various gas chromatographic counting geometries possible. The extent of degradation and a means of eliminating it are thus often important considerations in serum analyses.

Untreated chelates.-In order to measure the extent and effect of Szilard-Chalmers decomposition, separation was carried out with no attempt to repair the chelate irradiation damage. The result for all three Cr chelates was the same. When the injection ports and traps were visually examined after gas chromatographic separation, a small white residue remained in the injection port, while a large amount of green chelate had condensed in the trap. The bulk of the chelate had passed through the system intact. But when the ^{51}Cr content of the trap and injection port was measured, incomplete elution was found. Table 22 shows that 85-90% of the ^{51}Cr activity was found to have never left the injection port.

Table 22. Annealing Studies Recovery of Metal Chelates From Chromatographic System

Chelate	Treatment	Residual Activity, % of Total			
		Injection Port Liner	Column		Trap
			Column Packing	Teflon Tubing	
$Cr(hfa)_3$	none	89.1	4.6		6.3
$Cr(fod)_3$	none	84.1	9.8		6.1
$Cr(tfa)_3$	none	89.0	2.7		8.3
$Cr(tfa)_3$	10% Excess Ligand	11.1	35.8	0.25	52.9
$Cr(tfa)_3$	10% Excess Ligand, Annealed	2.80	2.97	0.70	93.5
$Cr(tfa)_3$	100% Excess Ligand Annealed	0.88	1.44	0.38	97.3
$Cr(tfa)_3$	Synthesized from Labeled Cr	0.35	1.36	0.38	98.16

What happened was that most of the chelate was totally unaffected by the irradiation. It eluted normally. But where ^{51}Cr neutron capture did occur, Szilard-Chalmers decomposition resulted in at least 85% of the ^{51}Cr atoms remaining in an unchelated form and thus not being eluted. It is clear that some form of effective treatment must be usef if valid data are to be obtained from postirradiation chelate separation techniques applied to irradiated metal chelates.

Treated chelates.-Table 22 also contains the results of the experiments involving treatment of the irradiated chelate solution. Only 10% excess ligand and no annealing beyond that which takes place in the reactor increases the eluted ^{51}Cr activity from 8% to 53%. This figure can be improved even more by annealing. The best and most consistent results come from combining annealing with the addition of 100% excess ligand. When these results are compared with those of an elution study involving chelate synthesis from ^{51}Cr labeled starting material, the nearly identical results indicate the completeness of radiation damage repair. The measures necessary to prevent loss of chelated radioisotopes in irradiated metal chelate solutions are thus both simple and effective.

Gas Chromatography

Those aspects of the gas chromatographic separation of metal chelates which apply to all inorganic gas chromatography are discussed in the Introduction. Two considerations which are not important in ordinary inorganic gas chromatographic analyses but which must be taken into account in radiochemical separations are the radiochemical purity of the eluate and the design of the radiation measurement system.

Incomplete Elution

Obtaining eluate with the proper radiochemical purity from a gas chromatographic metal chelate separation requires that the activity of each component remain unchanged during elution. The extent to which activated atoms of a given chelate are lost can be determined by measuring the amount of injected radioactive metal chelate which remains in the instrument after the sample has eluted.

Residue measurement.-The results of such measurements for several chelates are shown in Table 23. Efficient vaporization with minimal decomposition in the injection port is indicated for all chelates. However, the Teflon tube showed a small amount of chelate retention due to its length and thin liquid phase coating. The packing was the only system component which kept large amounts of chelate from eluting.

There is variation in elution behavior among the chelates. The $Cr(tfa)_3$ results were excellent. The higher column temperature used for $Cr(fod)_3$ may have contributed to decomposition,

Table 23. Percent Recovery of Metal Chelates in Chromatographic System

Chelate	Residual Activity, % of Total			
	Injection Port Liner	Column Packing	Teflon Tubing	Trap
$Cr(tfa)_3$	0.35	1.36	0.38	98.16
$Cr(fod)_3$	0.35	15.7	0.75	83.0
$Be(tfa)_2$	0.07	2.7	3.0	94.2
$Fe(fod)_3$	0.30	23.0	2.48	74.3
$Cu(tfa)_2$	1.30	23.0	3.5	72.2
$Gd(fod)_3$	1.0	35	1.3	63
$Lu(fod)_3$	0.51	7.0	0.43	92.1

as the high percentage on the column packing indicates. The $Be(tfa)_2$ results were similar to the $Cr(tfa)_3$ results. The $Gd(fod)_3$ data were not as accurate as those from the other experiments because the isotope being measured had gone through three half-lives before counting. This meant that there was a mixture of daughters and other isotopes whose half-lives were unknown, and no correction could be made among the activities measured during each experiment. However, it can be determined that nearly one third of the total activity was found in the column packing. This differs from the $Lu(fod)_3$ results, even though the same column and conditions were used, and both are rare earths. One contributing difference between the chelates lies in their metal ionic radii. Gd^{+3} is 15% larger than Lu^{+3}; ions with larger radii form chelates which elute at higher temperatures (172). Both the $Cu(tfa)_2$ and $Fe(fod)_3$ left large residues on the packing material. These may be reduced by raising the column temperature, but more extensive changes will probably be necessary before good quantitative results can be obtained with these chelates.

To summarize, $Cr(tfa)_3$, $Be(tfa)_2$, and $Lu(fod)_3$ with their respective columns are good choices for radiochromatography. The rest of the chelates will require modification of the chromatographic system described here before they too will be suitable for gas chromatographic separation in NAA.

The large residues in some cases would seem to indicate serious deficiencies in the application of gas chromatography

to the quantitative analysis of these metal chelates. This is not necessarily the case. Many workers report the necessity of "conditioning" a freshly prepared column by injecting samples of the particular chelate to be analyzed until reproducible elution is obtained (148, 317). Some laboratories even find this procedure necessary after the system has been idle for only a few hours. This allows quantitative analysis to be performed in spite of the incomplete elution of the first few injections in a long series.

If gas chromatography is to be successfully applied to NAA, it may not be sufficient merely to "condition" the column. In a stable isotope experiment the nuclear properties of different samples are the same. If there is exchange between a particular analyte injection and atoms injected prior to analysis for conditioning purposes there is no adverse effect. If, however, there is exchange between two radioisotope injections which have different specific activities, activity measurement of the eluate will yield erroneous results. The exchange experiment with $Cu(hfa)_2$ showed cross contamination to be occurring. The stable sample, injected several hours after the irradiated $Cu(hfa)_2$ sample, eluted carrying with it ^{64}Cu from the previous injection. This particular system, then, would not be suitable for carrying out radioactive $Cu(hfa)_2$ separation.

Injection procedures.-Solid injection, liquid injection, and DMCS treatment were evaluated in an effort to promote complete elution. The solid sampling device used in the

$Cu(hfa)_2$ study performed well. A solid sampler allows the injection of a relatively large quantity of chelate without a large amount of solvent which would be detrimental to the separation and interfere with effluent trapping. Also, the sealed quartz ampoule can be taken from the reactor and placed directly in the sampling device without the handling of open containers and evaporation involved in liquid sampling. The measurements of activity from the solid sampler and quartz fragments showed large amounts of metal chelate residue. This was partly due to Szilard-Chalmers decomposition. The intact visible chelate residue was present because of low injection port temperatures and incomplete carrier gas purging of the inside of the barrel of the solid sampling device.

The length of the quartz ampoules used with the solid sampler was found to be critical. If the ampoule was too short, it was not broken during sampling; instead, it was forced upward, breaking the glass injection port liner. If the ampoule was too long, the large amount of broken quartz fragments prevented all the sample from leaving the sampler as a single narrow band. Lengths between 0.406 in and 0.419 in worked best.

Syringe injection of solutions is advantageous when the volume of solvent necessary to dissolve a useful amount of chelate can be tolerated. The extract from a chelation reaction can be introduced directly with no processing or sealing. Furthermore, syringe injection does not entail cleaning quartz fragments from the injection port or

interrupting the carrier flow each time an injection is made. Sometimes excess solvent problems can be eliminated by injecting a relatively large amount of sample into a cold column. After the solvent has eluted, the oven heat is quickly increased, vaporizing the chelate without broadening sufficient to prevent separation.

The treatment of the injection port and column with dimethyldichlorosilane (DMCS) showed little effect on the quantity of chelate eluted. Figures 12 and 13 show the result of the DMCS treatment. The chelate peak areas of the 1-µl injections are plotted on the vertical axis; the horizontal axis represents order of injection. Peak area averages for all the injections made at the same stage of DMCS treatment are also plotted. There is no significant increase in slope with treatment. This indicates DMCS treatment is of no value in decreasing incomplete elution in this case.

Counting Geometry

After the radioactive metal chelate has eluted, it must be held in a reproducible manner in front of a radiation detector long enough to allow good counting statistics and detection of low activity components. Factors to be considered in choosing a counting geometry are reproducibility and ease of restoration before a subsequent sample arrives to be counted.

The relative response of the NaI(Tl) crystal as a function of sample position is an important consideration in the design of counting geometries. Consecutive samples need to be held

Figure 12. Effect of DMCS Treatment on $Cr(fod)_3$ Elution.

● = peak areas

▲ = peak area averages

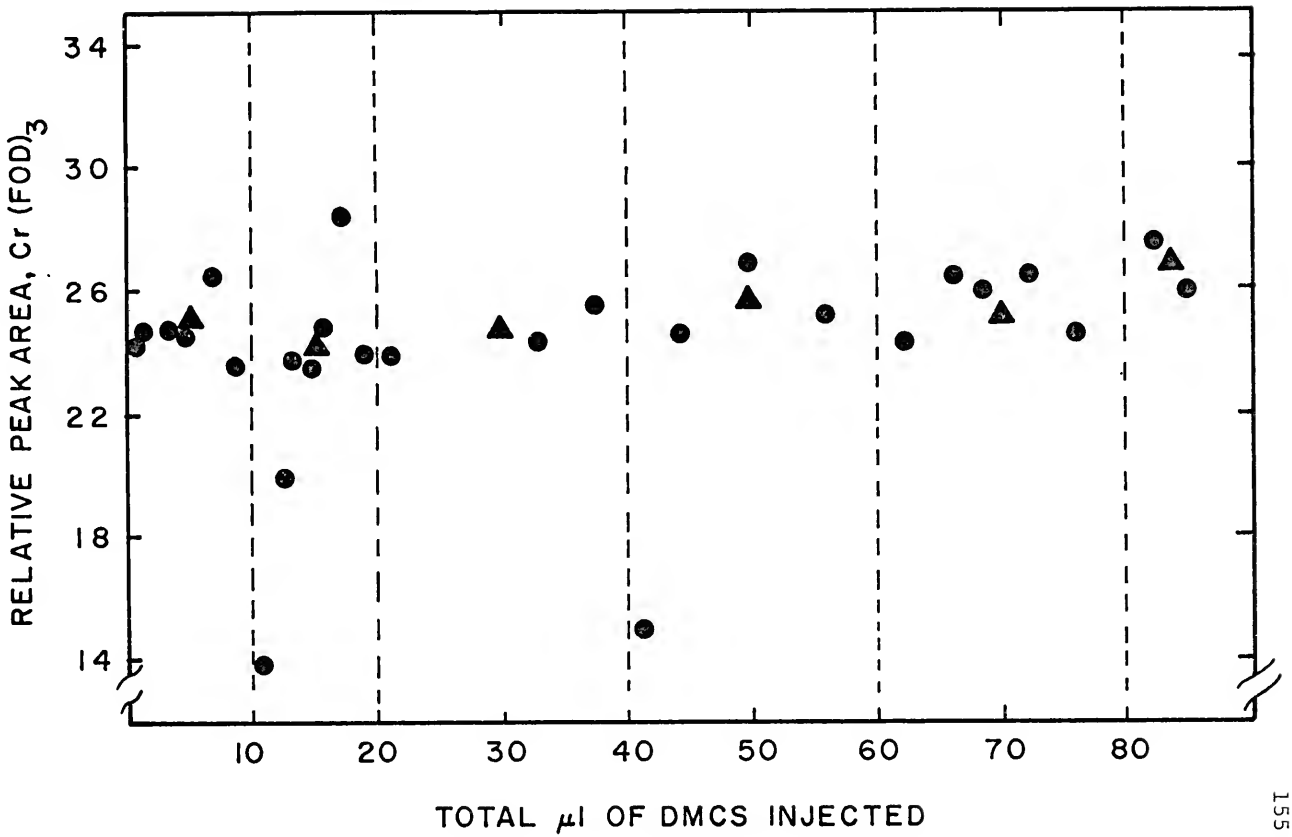
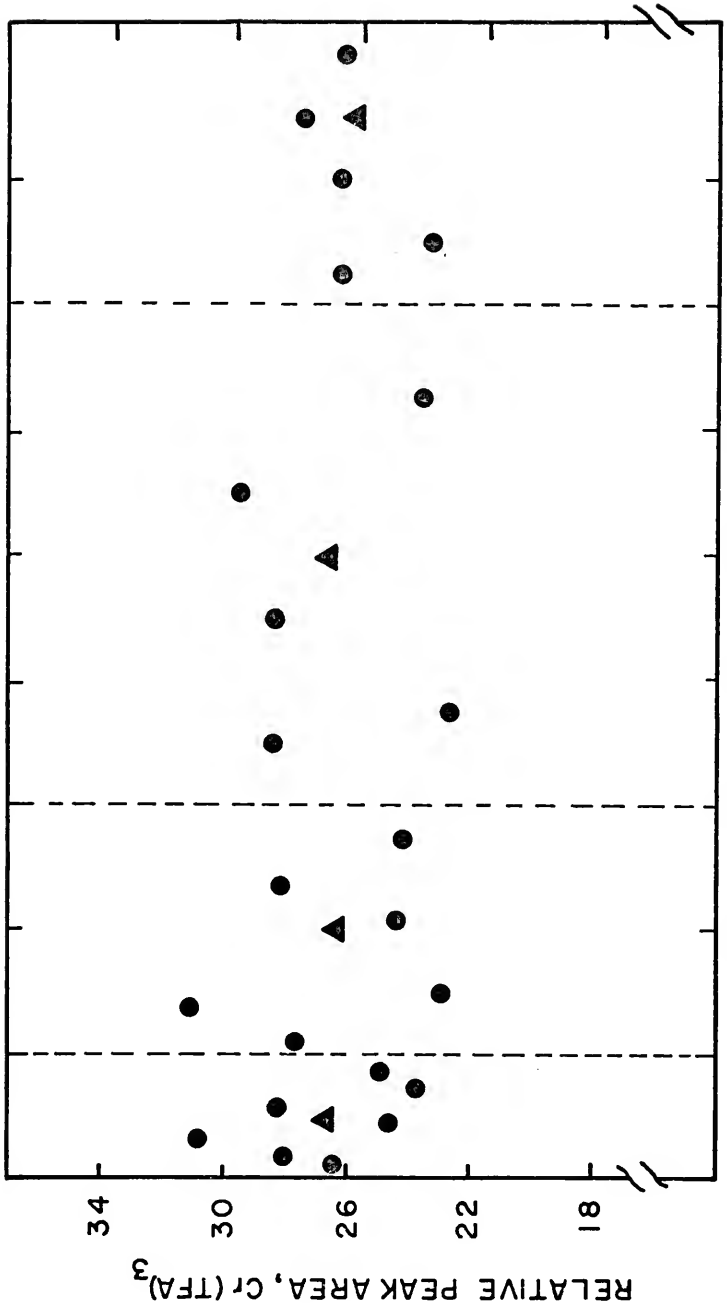

RELATIVE PEAK AREA, Cr (FOD)3
34
30
26
22
18
14
10
20
30
40
50
60
70
80
TOTAL μl OF DMCS INJECTED

Figure 13. Effect of DMCS Treatment on $Cr(tfa)_3$ Elution.

● = peak areas

▲ = peak area averages


RELATIVE PEAK AREA, $Cr(TFA)_3$
34
30
26
22
18
10
20
30
40
50
60
70
80
TOTAL μl OF DMCS INJECTED

in regions with the same response if reproducible results are to be obtained. Figure 14 shows the plot of response versus position obtained by slowly passing a ^{137}Cs source across the face of the detector. The region where the response decreased by no more than 10% from the maximum was found to be a 33-mm diameter circle concentric with the cylindrical detector. The active part of each geometry was kept within this region.

Stainless steel spiral.-The stainless steel geometry can yield a differential radiochromatogram with characteristics similar to a conventional chromatogram. Figure 15 shows such a chromatogram obtained from ^{64}Cu enriched $Cu(hfa)_2$ which had been separated from an irradiated $Cu(hfa)_2$-$Cr(hfa)_3$ mixture. The tailing is pronounced, indicating either adsorption or condensation on the walls of the counting geometry tubing.

The only way to obtain good counting data with this geometry is to stop the flow of carrier gas while counting. Otherwise, the residence time of the radionuclide in the geometry is too short. Figure 15 shows that the peak maximum is only one or two channels wide. In this case the maximum counting time would be 2.4 s. Since stopping the flow at the optimum time would require precise timing as well as an extremely stable chormatographic system, this type of geometry is not best for quantitative analysis of low-activity radionuclides.

Figure 14. Response of the NaI(Tl) Detector as a Function of Position. The Two Curves Represent Response on Two Radii Oposite Each Other.

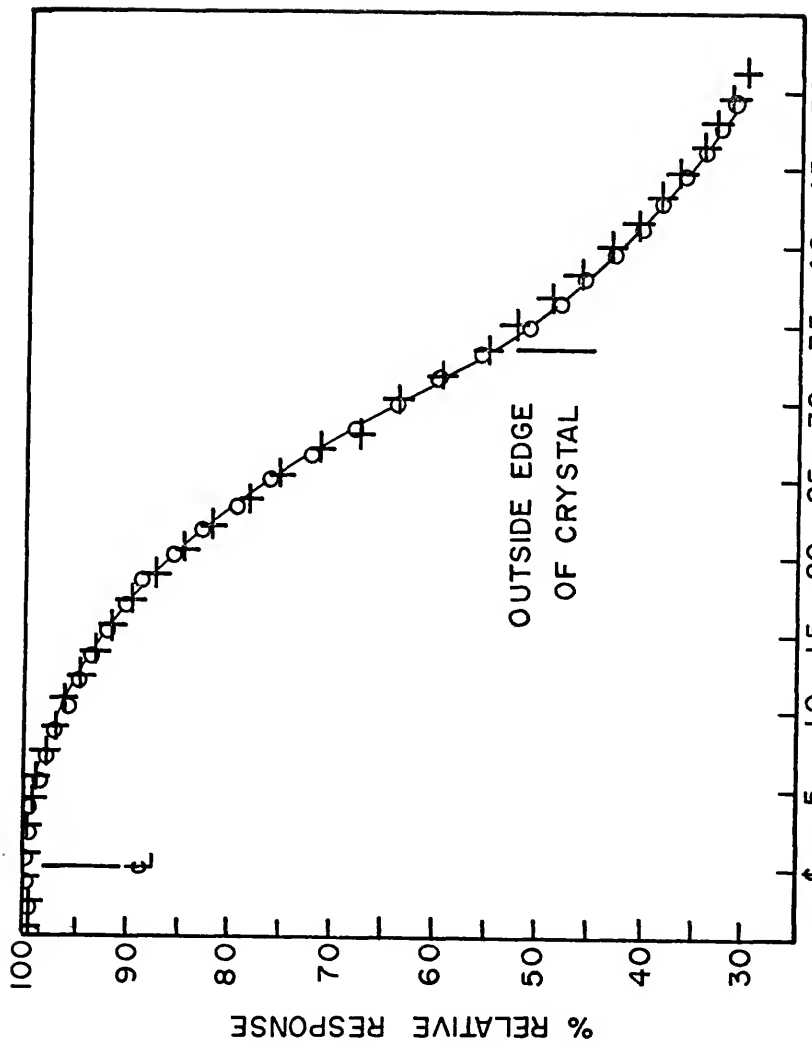
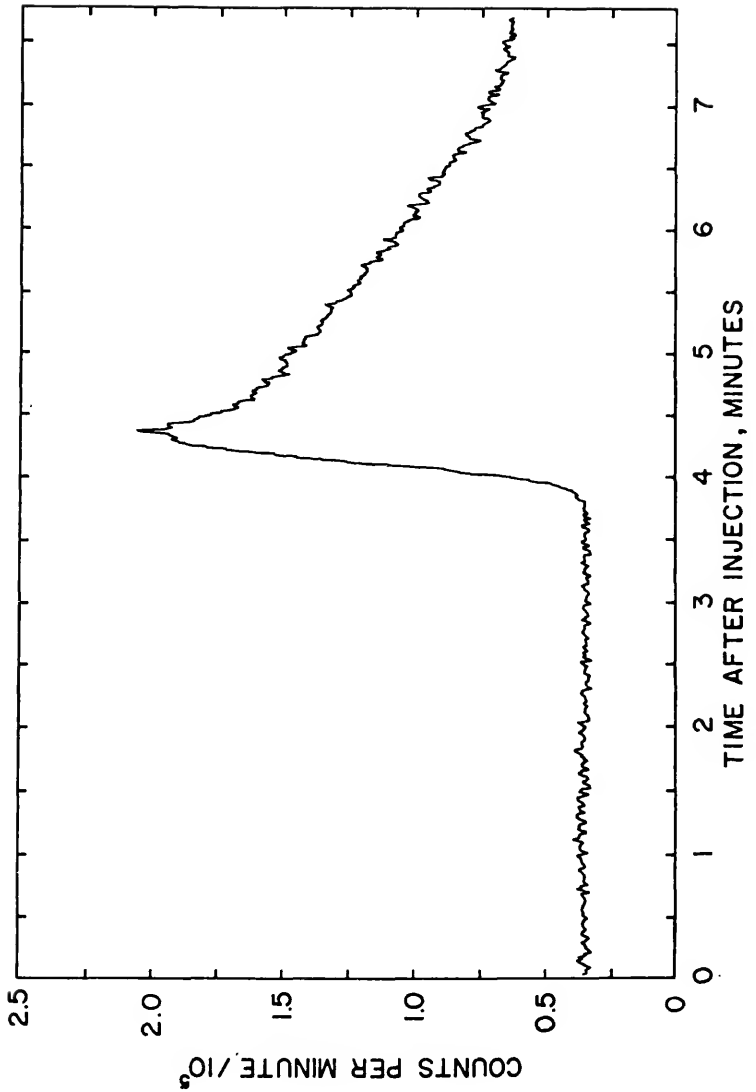

% RELATIVE RESPONSE
100
90
80
70
60
50
40
30
℄
OUTSIDE EDGE
OF CRYSTAL
℄ 5 10 15 20 25 30 35 40 45 50
DISTANCE FROM CENTER OF CRYSTAL, MM.

Figure 15. $Cu(hfa)_2$ Activity Counted in a Stainless Steel Flow-Through Counting Geometry After Separation From a $Cu(hfa)_2$-$Cr(hfa)_3$ Mixture.


COUNTS PER MINUTE / 10³
2.5
2.0
1.5
1.0
0.5
0
0
1
2
3
4
5
6
7
TIME AFTER INJECTION, MINUTES

Charcoal trap.-This was by far the simplest and least susceptible to error of all the geometries tested. Moreover, counting the activity from the traps can be done in a location remote from the gas chromatograph and free from its attendant background. Traps from a multicomponent separation of short half-life radionuclides can be counted simultaneously, thus maintaining maximum sensitivity and minimum decay correction uncertainty. The data for the incomplete elution experiments were taken in this way. There were no problems associated with this method of trapping.

Reversible counting geometry.-The most elegant counting geometry constructed was the reversible counting geometry packed with column packing. It combines the advantages of the spiral flow-through and the charcoal trap, yet has none of their disadvantages. Like the charcoal trap, the reversible geometry holds the radionuclide for an indefinite counting time. Unlike the flow-through type, however, the timing of the carrier gas flow stop is not critical, since the sample moves along the packed tube much more slowly than through empty tubing. The reversible geometry does not hold the sample permanently. After the count is finished, heat and purge gas are applied to flush the geometry. After it is cool again, it is ready for the next component of the sample to be eluted and trapped. Here is complete flexibility, indefinite counting time, freedom from critical stop flow timing, and, ideally, complete reversibility.

Figure 16 shows a 400 channel radiochromatogram which illustrates the reversibility of the geometry. A solid $Cu(hfa)_2$ sample was injected. The activity suddenly rises as the radioactive plug enters the geometry. Since flow was not stopped, the tail of the chromatographic peak accumulates and the count rate slowly increases. At channel 364, heat was applied to the geometry and the temperature started to rise from 60 °C at about 20 °C per minute. The trapped chelate started to move as the geometry began to heat. First it moved to a more sensitive counting position, as indicated by the steep rise in count rate. Then the rate drops to the background level as the radioactive material is flushed from the geometry.

The performance of the improved reversible counting geometry can be evaluated using the two 400-channel radiochromatograms in Figure 17. The left-hand portion follows the activity increase as the radioactive chelate accumulates in the packed region. Some tailing is present, but after 12 min the activity remains constant. Fifteen minutes after injection, the flow was stopped for an extended period to permit γ-ray counting under static conditions. The half-life of the radioisotope is the only parameter which limits counting time. The right-hand portion of the curve shows the chelate being purged from the geometry. The two curves have plateaus at the same activity levels, indicating no change in the measured activity while the flow was stopped, and a complete purge with background activity returning to the

Figure 16. Radiochromatogram Illustrating the Counting of $Cu(hfa)_2$ in the Reversible Counting Geometry.

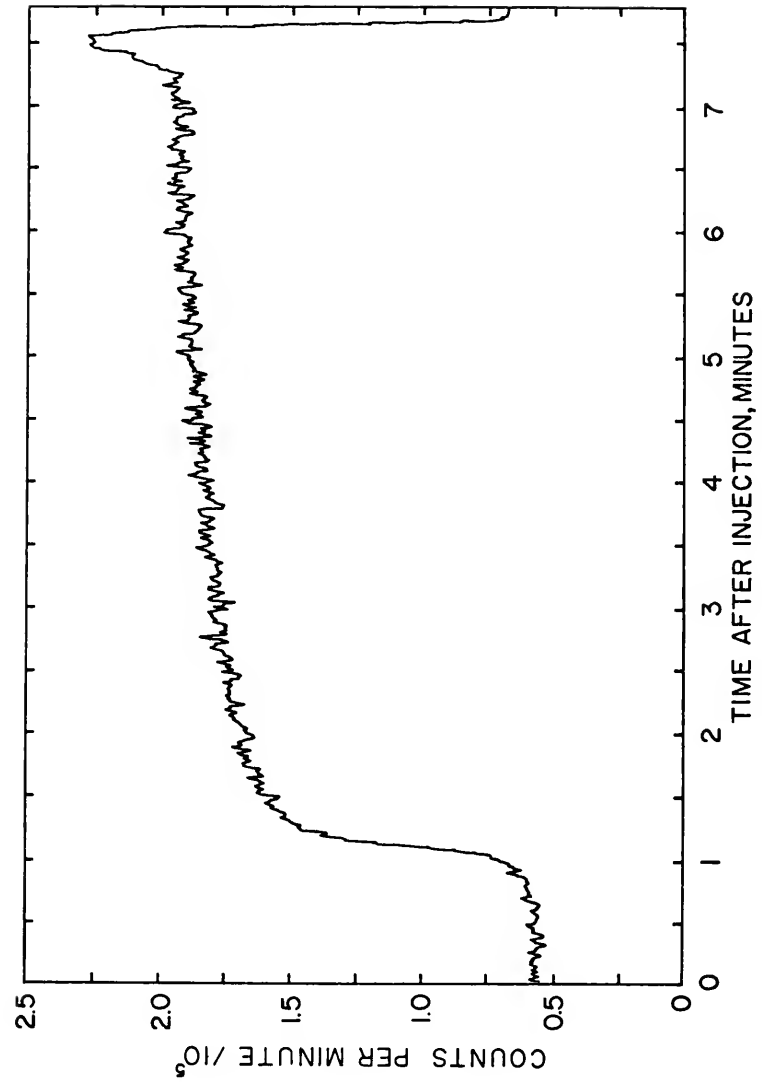
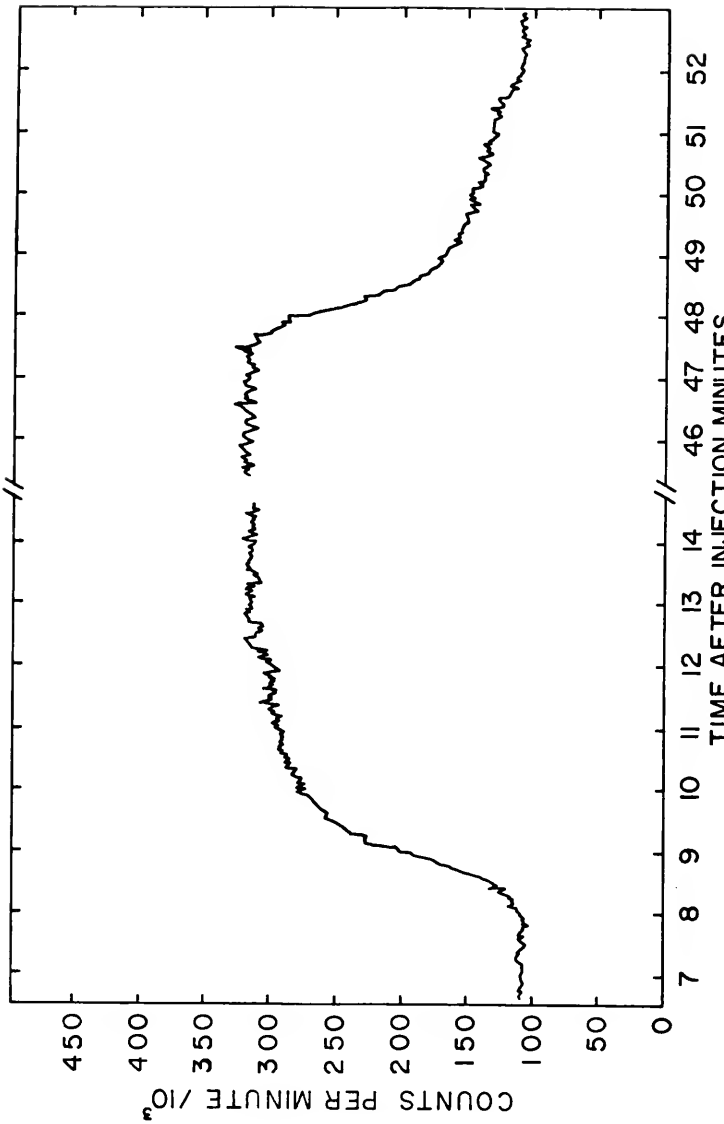

COUNTS PER MINUTE /10^6
2.5
2.0
1.5
1.0
0.5
0
0
1
2
3
4
5
6
7
TIME AFTER INJECTION, MINUTES

Figure 17. Radiochromatogram Illustrating Operation of the Improved Reversible Counting Geometry.


COUNTS PER MINUTE /10²
450
400
350
300
250
200
150
100
50
0
7
8
9
10
11
12
13
14
46
47
48
49
50
51
52
TIME AFTER INJECTION, MINUTES

same level as before injection. The improved circular design with its extended region of uniform detector sensitivity eliminated the sensitivity change which caused the sharp peak at the far right of Figure 16. Lack of uncertainty in sample position sensitivity results in better reproducibility with this design.

Similar results were obtained with $Cr(tfa)_3$. Samples with activities of several hundred counts per minute were successfully counted and purged from the system. Dependence of photopeak area on injected sample volume was also established.

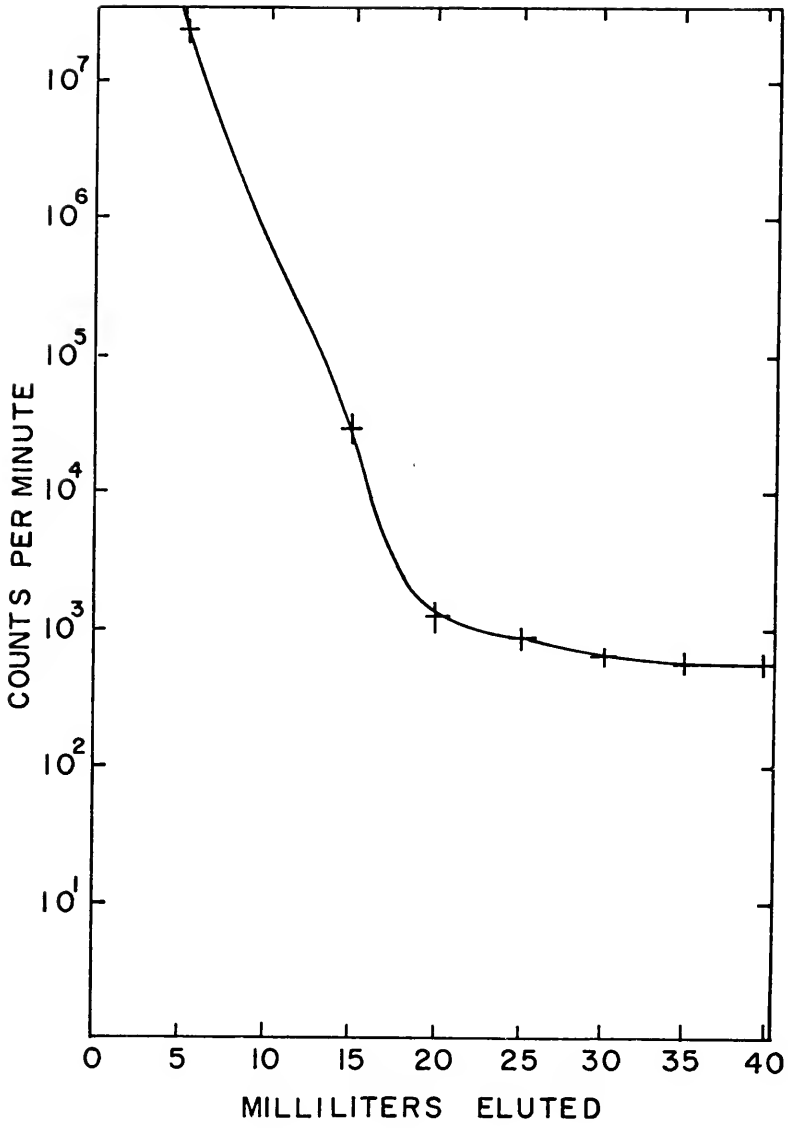
Ion Exchange

Chelating ion exchange resins such as Chelex-100 are potentially quite useful for sodium removal in serum trace element analysis. The strong bond between the metal and the chelating resin enables extraction from very dilute solutions of high ionic strength such as dissolved serum ash. Rapid, quantitative sodium and potassium removal from a large number of serum constituents by this method should be extremely valuable in NAA.

Postirradiation Separation

Sodium separation optimization.-The sodium separation is optimized when the minimum amount of rinse water necessary to remove all the interfering ^{24}Na has been eluted from the column. At this point, sodium removal is adequate, while trace element loss is minimized. The sodium removal process can be followed in Figure 18. The total activity of each 5-ml portion of eluate decreased rapidly during rinsing; after 25 ml had been eluted a sodium decontamination factor of 10^6 had been achieved and very little ^{24}Na was present in the eluate. However, ^{56}Mn and ^{82}Br were also present. The next three 5-ml eluate samples each showed ^{24}Na and ^{82}Br activity at equal levels. Both the ^{24}Na and ^{82}Br activities decreased as rinsing continued. In this case, a compromise between total ^{24}Na removal and total trace element retention must be made. In view of the sudden drop in the contamination rate around 25 ml, less rinsing is not advisable. However,

Figure 18. ^{24}Na Content of Eluate as Elution From Chelex-100 Proceeds.


COUNTS PER MINUTE
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0
5
10
15
20
25
30
35
40
MILLILITERS ELUTED

additional rinsing is beneficial only if the radionuclide of interest will not be removed along with the ^{24}Na.

Serum analysis.-The absence of a blank in postirradiation separations is especially appealing in ion exchange. Another advantage in irradiating before an ion exchange separation such as this is that no final elution step is necessary. The activity measurement can be made while the radioactive trace metals are still on the resin, thus elimiating possible loss during elution.

The 1-hr γ-ray spectrum recorded 2.5 hr after a 30-min irradiation showed moderate amounts of ^{24}Na, ^{82}Br, and ^{38}Cl activity, along with small amounts of ^{128}I, ^{140}La, ^{56}Mn, and ^{64}Cu activity in the resin. The measurement was made while the radioactive metals chelated by the resin were still on the column. No elution had taken place, except for rinsing the sodium from the resin.

Preirradiation Separation

Short irradiation.-The 3-hr irradiation time used in this experiment gives the sample a much greater neutron exposure than the 30-min irradiations used with postirradiation separations, yet it is not so long that turnaround time for the determination is inordinately long or that there is unnecessary delay in the determination of nuclides which reach saturation quickly. The purpose of the experiment was to evaluate this intermediate time period as well as find out more about the applicability of this type of separation to trace metal analysis in serum.

NAA of the resins after complete elution showed that the same elements were present in the resin blank and the resin used in the separation experiment. Apparently none was lost in the separation due to permanent retention on the resin. The blank containing the reagents used in the separation did contain a significant amount of potassium. There was enough to obscure small photopeaks which might otherwise have been detected. The potassium apparently came from the KOH used to adjust the pH of the ash solution, even though all potassium should have been removed during the sodium separation. Ammonium hydroxide could have been substituted for KOH, but some ammine complex sequestration of the trace metals might have occurred. The best results are obtained using KOH along with thorough removal of alkali metal ions.

The radionuclides detected in the irradiated effluent containing the trace metals were ^{24}Na, ^{42}K, ^{69m}Zn, ^{38}Cl and ^{56}Mn. More would undoubtedly have been found if a higher flux had been used. The 3-hr irradiation showed little improvement over the 30-min irradiation as far as the number of elements detected. Of course, the activities of the radionuclides detected were greater, and therefore more accurate quantitative data could be determined.

Long irradiation.-When used with a Chelex-100 separation, a long irradiation yields a much greater number of detectable radionuclides than do the shorter irradiation times discussed in previous sections. For survey work, or for determining the concentrations of trace elements with long half-lives, a long irradiation is necessary.

Figure 19 shows the γ-ray spectrum of one of the three serum samples which underwent ashing, Na separation using Chelex-100, and irradiation for 52 hr. This 15-hr count followed a 7-d decay. The radionuclides identified are listed in Table 24. The solution blank contained a small amount of ^{51}Cr and ^{60}Co. ^{65}Zn and ^{198}Au were present in significant amounts in the blank, although none of the metals found in the serum originated entirely in the blank.

Of all the various procedures used here for the ion exchange separation of sodium from the trace elements in serum, the preirradiation separation with a long irradiation yielded the best results in terms of the number of elements detected. A postirradiation separation following a neutron exposure time of several hours may also be productive, but remote handling of the sample will be required.

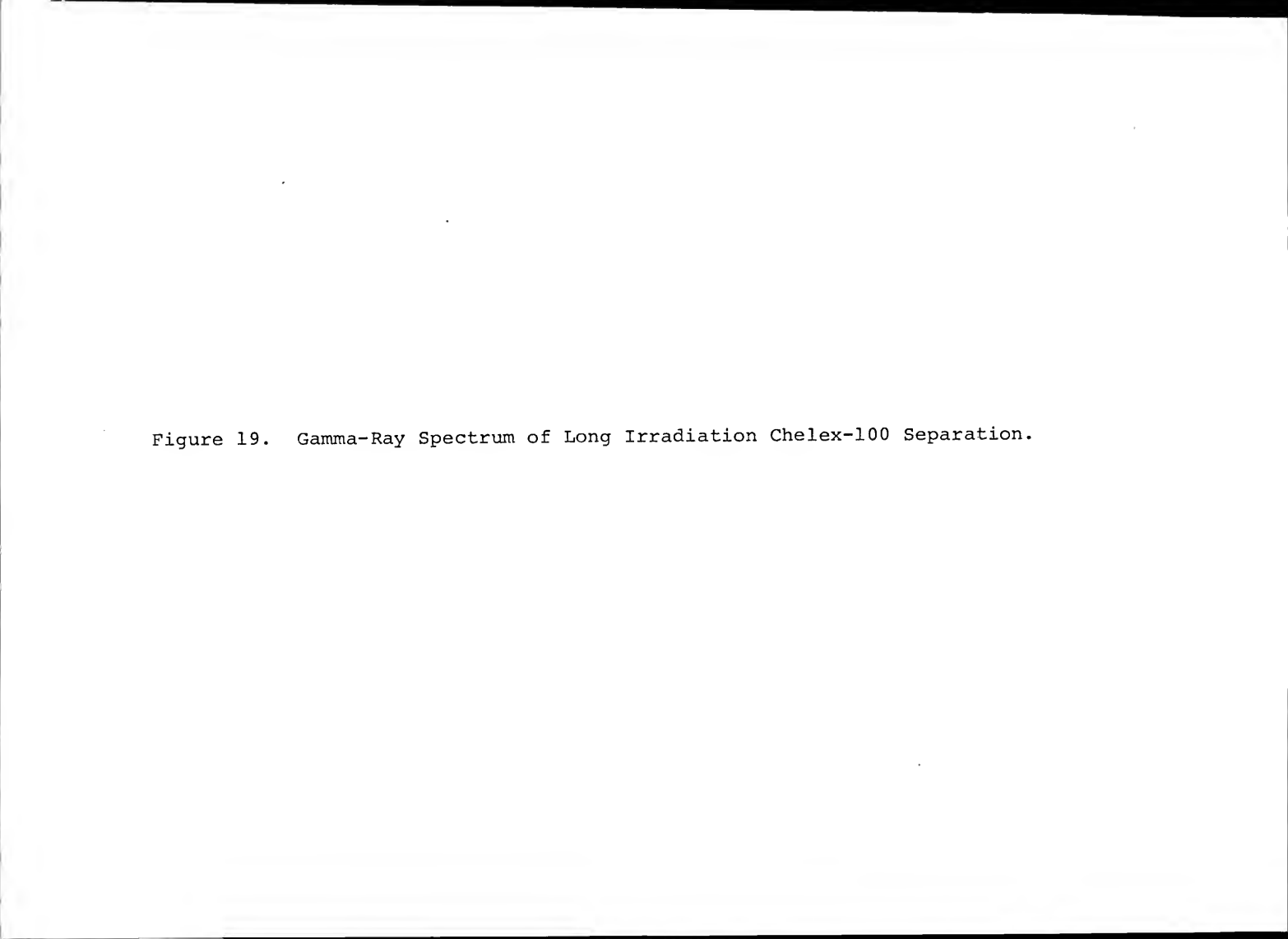


Figure 19. Gamma-Ray Spectrum of Long Irradiation Chelex-100 Separation.

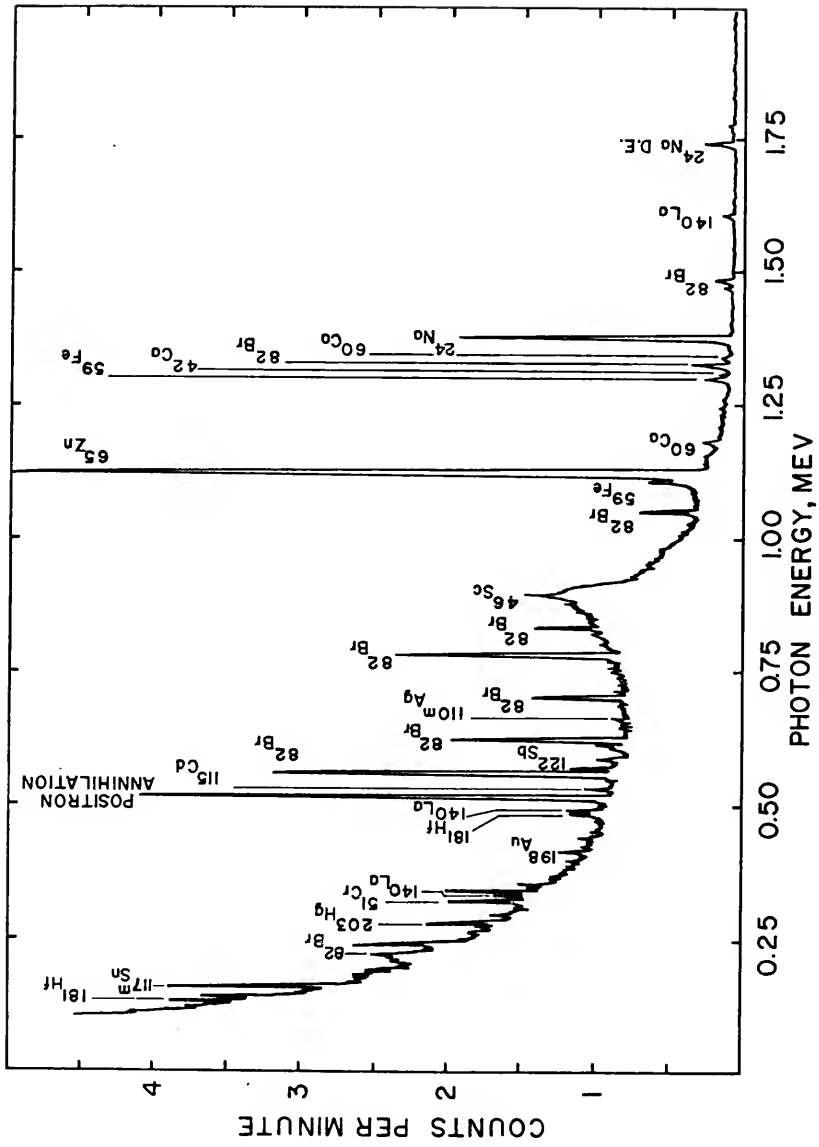

COUNTS PER MINUTE
PHOTON ENERGY, MEV
0.25
0.50
0.75
1.00
1.25
1.50
1.75
1
2
3
4
24Na D.E.
140La
82Br
24Na
60Co
82Br
42Ca
59Fe
65Zn
60Co
59Fe
82Br
46Sc
82Br
82Br
110mAg
82Br
122Sb
82Br
82Br
115Cd
POSITRON ANNIHILATION
140La
181Hf
198Au
140La
51Cr
203Hg
82Br
117mSn
181Hf

Table 24. Long Irradiation Chelex-100 Separation

Isotope	γ-Ray Energy, keV (315)	Isotope	γ-Ray Energy, keV
^{181}Hf	133.1	^{82}Br	698.3
^{193}Os	139.0	^{82}Br	776.6
^{117m}Sn	158.4	^{82}Br	827.8
^{82}Br	222	^{46}Sc	889.4
^{203}Hg	279.1	^{82}Br	1043.9
^{51}Cr	320.0	^{59}Fe	1098.6
^{140}La	328.6	^{65}Zn	1115.4
^{59}Fe	335	^{60}Co	1173.1
^{198}Au'	411.8	^{59}Fe	1291.5
^{181}Hf	482.2	^{47}Ca	1296.9
^{140}La	486.8	^{82}Br	1317.2
^{115}Cd	527.7	^{60}Co	1332.4
^{82}Br	554.3	^{24}Na	1368.4
^{122}Sb	564.3	^{82}Br	1474.7
^{82}Br	619.0	^{140}La	1595.4
^{110m}Ag	657.8		

Conclusions

Sequence of Operations

Figure 20 shows the procedural options which are available when the separation techniques evaluated here are used in NAA of trace elements in serum. The two fundamental choices are which sodium separation technique to use and when to irradiate.

Preirradiation separation.-The primary advantage in sodium removal before irradiation is that after leaving the reactor the sample needs no further processing before counting. This avoids decay of short half-life radioisotopes during separation and eliminates personnel exposure to the high radiation levels present after sodium irradiation. The latter is quite important, since irradiation of untreated serum is limited to a maximum of 30 min unless special shielding and sample manipulating equipment are used.

Postirradiation separation.-The chief advantages in delaying separation until after irradiation are the option of carrier addition and the elimination of the reagent blank. Thus, in situations where both the irradiation and separation can be carried out quickly, a postirradiation separation is more effective.

When choosing the best sequence of operations for determining the serum concentrations of a particular element, its nuclear properties, particularly half-life, are considered. Each element which can be determined using NAA has isotopes

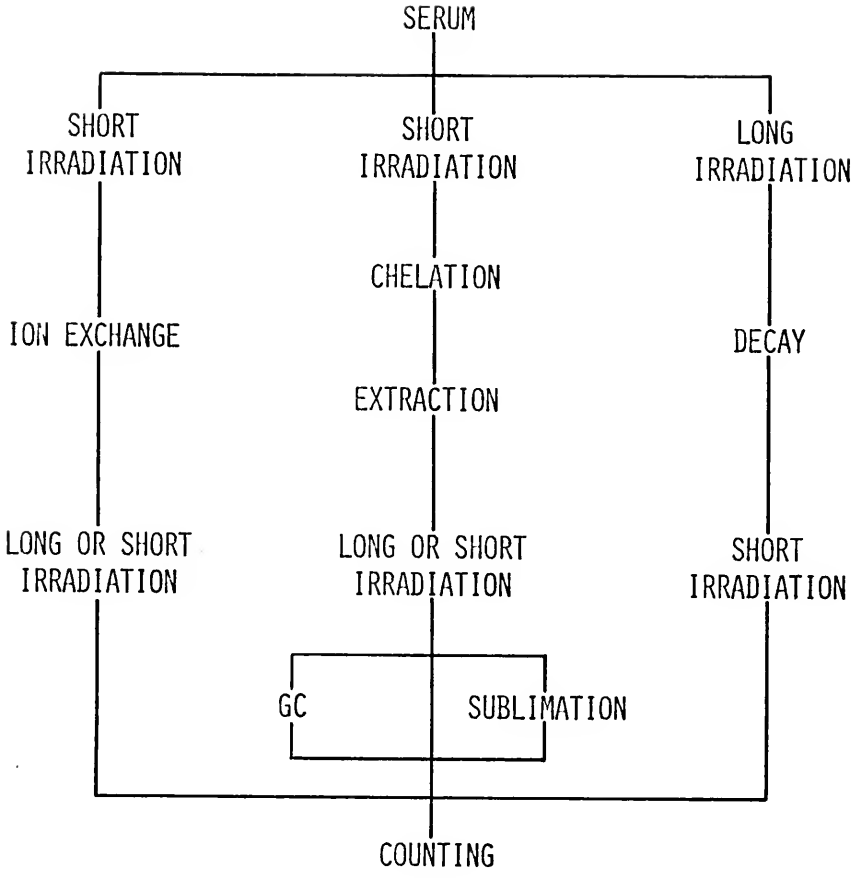


Figure 20. Procedural Options

which can be placed into at least one of the four categories listed in Table 25. However, a wide range in half-lives may prevent simultaneous analysis of certain combinations of elements.

Separation Techniques

Once the length of the irrradiation and its location in the analysis scheme have been established, the separation technique options can be evaluated.

Chelation.-Both the chelation from solid ash and the chelate extraction from solution worked well. Each is effective in removing ^{24}Na contamination and permitting further separation if needed. Although the better pH control of the ash solution allows selective chelation of some metals and thereby gives greater selectivity, direct chelation from the solid ash is simpler and much faster. The failure of the chelate extraction from solution procedure to yield a large number of elements when used after irradiation is due to the delay in counting caused by the additional sample manipulation. Such procedures must be restricted to preirradiation separations.

Chelate separation.-Both gas chromatography and the vapor-phase separation techniques were effective. GC has much greater resolution, but the increased throughput and lack of column retention make the simpler method valuable too. The trapping systems, which can be used with either method, were satisfactory. A charcoal trap or an open Pyrex tube at room temperature work best where the sample is not complex and the analysis time is not critical. There is

Table 25. Procedure Options Grouped by Half-Life

I	II	III	IV
Elements sufficiently activated before ^{24}Na radiation level interferes.	Elements sufficiently activated before ^{24}Na radiation level becomes hazardous.	Elements requiring more than 30 min of irradiation and which decay before ^{24}Na drops to a noninterfering level	Elements with half lives much longer than that of ^{24}Na.
Very short irradiation. Pneumatic rabbit.	Irradiation shorter than 30 min.	Irradiation lasting from 30 min to several days.	Long irradiation and long decay.
No separation.	Pre- or postirradiation separation required.	Preirradiation separation required.	No chemical separation required.
Example: 11 s ^{20}F.	Example: 2.58 hr ^{56}Mn.	Example: 12.8 hr ^{64}Cu.	Example: 5.26 y ^{60}Co.

more potential versatility with the reversible geometry. It makes γ-ray counting for a series of eluted components from a complex sample quite simple.

The information about chelate retention by the gas chromatographic system is applicable to trace element analysis as well as to radiochemical separations in NAA. Some chelates are eluted almost completely and some are not. When a chelate injection is not entirely eluted from a fresh column, exchange with the species needed to condition the column for complete elution can preclude the system's use in NAA. Before such a separation is attempted, this aspect must be considered.

Ion exchange.-The Chelex-100 separation yielded excellent ^{24}Na decontamination rapidly with a minimum amount of sample manipulation. It was the only method evaluated here which was suitable for postirradiation separation. The trace metal resolution after sodium removal is poor, especially when compared to chelate methods involving further separation using GC. However, when high-resolution counting equipment is available, chelating ion exchange separations can yield resolution sufficient for a wide range of determinations.

Future Work

Automating the experiment will increase the accuracy, the speed, and the number of elements analyzed. Remote sample handling will allow postirradiation separation to be used most effectively. On-line computer control of the experiment will allow such improvements as eluent volume optimization

by continuous effluent monitoring in ion exchange separations. The reversible counting geometry and GC stop-flow system were designed for such automated operation.

Szilard-Chalmers reactions in the naturally occurring complexes in serum can replace ashing. Ligands forming highly stable complexes can be added to serum before irradiation; chelates with very high specific activities can then be extracted after irradiation.

Metal complex separation using liquid chromatography permits the use of thermally unstable complexes. The wider range of ligands available can make multielement quantitative separation of a greater number of elements possible.

REFERENCES

1. W.G. Hoekstra, Ann. N.Y. Acad. Sci., 199, 182 (1972).

2. J.T. McCall, N.P. Goldstein, and L.H. Smith, Fed. Proc., 30, 1011 (1971).

3. M.A. Schroeder, J. Chron. Dis., 18, 217 (1965).

4. W. Mertz, Ann. N.Y. Acad. Sci., 199, 191 (1972).

5. K.G. Kjellin, Int. J. Appl. Radiat. Isotopes, 15, 461 (1964).

6. W.F. Beyer, Clin. Chim. Acta, 38, 119 (1972).

7. D. Kolihova and V. Sychra, Anal. Chim. Acta, 36, 479 (1973).

8. I.L. Liplavk and L.N. Ushkova, Lab. Delo, 7, 399 (1969); Chem. Abstr., 71, 78049h (1969).

9. G.S. Fell, H. Smith, and R.A. Howie, J. Clin. Pathol., 21, 8 (1968).

10. G.C. Battistone, E. Levri, and R. Lofberg, Clin. Chim. Acta, 30, 429 (1970).

11. J.D. Khandekar, D. Mukerji, and G.C. Sepahn, Indian J. Med. Sci., 26, 813 (1972).

12. F.B. DeJorge, J. Sampaio Goes, Jr., J.L. Guedes, and and A.B. DeUlhoa Cintra, Clin. Chim. Acta, 12, 403 (1965).

13. T. Anovski, T. Memedovic, and M. Rastvovac, J. Radioanal. Chem., 12, 483 (1972); Chem. Abstr., 78, 107858y (1973).

14. E.L. Kanabrocki, T. Fields, C.F. Decker, L.F. Case, E. B. Miller, E. Kaplan, and Y.T. Oester, Int. J. Appl. Radiat. Isotopes, 15, 175 (1964).

15. E.L. Kanabrocki, L.F. Case, L. Graham, T. Fields, E.B. Miller. Y.T. Oester, and E. Kaplan, J. Nucl. Med., 8, 166 (1967).

16. E.L. Kanabrocki, L.F. Case, T. Fields, L. Graham, E.B. Miller, Y.T. Oester, and E. Kaplan, in "Proceedings of the 1965 International Conference on Modern Trends in Activation Analysis," College Station, Texas, 1965, p. 220.

17. A. Jacobson, S. Brar, T. Fields, I.G. Fels, E. Kaplan, P. Gustafson, and Y.T. Oester, J. Nucl. Med., 2, 289 (1961).

18. M.C. Haven, G.T. Haven, and A.L. Dunn, Anal. Chem., 38, 141 (1966).

19. D.A. Olehy, R.A. Schmitt, and W.F. Bethard, J. Nucl. Med., 7, 917 (1966).

20. W.F. Bethard, D.A. Olehy, and R.A. Schmitt, in "L'Analyse par Radioactivation et ses Applications aux Sciences Biologiques," Presses Universitaires de France, 1964, p 379; Chem. Abstr., 63, 3305g (1965).

21. W.F. Bethard, R.A. Schmitt, and D.A. Olehy, Strahlentherapie Sonderb., 5, 222 (1962); Chem. Abstr., 61, 9766c (1964).

22. N.A. Dubinskay, H.H. Mihelsons, and L. Pelekis, Neitronoaktiv. Anal., Akad. Nauk Latv. SSR, Inst. Fiz., 109, 1966.

23. D. Comar, G. Girault, and C. Kellershohn, Bull. Soc. Chim. Fr., 1, 1766 (1962).

24. THAI Office of Atomic Energy for Peace, Bangkok, Report THAI-AEC-22, 1969.

25. T. Asai, E. Meren, M. Okumoto, Y. Sato, and T. Azuma, Annu. Rep. Radiat. Center Osaka Prefect., 7, 99 (1966); Chem. Abstr., 67, 105916q (1967).

26. D. Brune, B. Frykberg, K. Samsahl, and P.O. Wester, "Determination of Elements in Normal and Leukemic Human Whole Blood by Neutron Activation Analysis," AE-60, Aktiebolaget Atomenergi, Stockholm, Sweden, 1961; Chem. Abstr. 59, 12013h (1963).

27. W.A. Wolstenholme, Nature, 203, 1284 (1964).

28. C.C. Thomas, Jr., G.P. Tercho, and J.A. Sondel, Nucl. Appl., 3, 53 (1967); Chem. Abstr., 66, 92354g (1967).

29. C.C. Thomas, Jr., G.P. Tercho, and J.A. Sondel, Trans. Amer. Nucl. Soc., 9, 69 (1966).

30. M.M. Molokhia and B. Portnoy, Br. J. Dermatol., 83, 376 (1970).

31. L.O. Plantin and P.O. Strandberg, Acta Rheumatol. Scand., 11, 30 (1965); Chem. Abstr. 63, 15362e (1965).

32. S. Meret and R.I. Henken, Clin. Chem., 17, 369 (1971).

33. C. Pfeiffer, Rev. Can. Biol., 31, Suppl. 73 (1972).

34. S. Sprague and W. Slavin, At. Absorption Newslett., 4, 228 (1965).

35. J.P. Matousek and B.J. Stevens, Clin. Chem., 17, 363 (1971).

36. D.M. Taylor, Biochem. J., 91, 424 (1964).

37. S. Giovannetti, Q. Maggiore, and R. Malvano, in "Nuclear Activation Techniques in the Life Sciences," Proceedings of a Symposium, Amsterdam, 1967, IAEA, Vienna, 1967, p. 511.

38. D. Brune, K. Samsahl, and P.O. Wester, "The Amounts of Arsenic, Gold, Bromine, Copper, Iron, Molybdenum, Selenium, and Zinc in Normal and Uremic Human Whole Blood. A Comparison by Means of Neutron Activation Analysis," AE-134, Aktiebolaget Atomenergi, Stockholm, Sweden, 1964; Chem. Abstr. 60, 14956c (1964).

39. D. Brune, K. Samsahl, and P.O. Wester, Clin. Chem. 13, 285 (1966).

40. W. Niedermeier and J.H. Griggs, J. Chron. Dis., 23, 527 (1971).

41. R. Malvano, U. Rosa, and P. Grosso, Int. J. Appl. Radiat. Isotopes, 18, 121 (1967).

42. K. Kasperek, H. Schicha, V. Siller, and L.E. Feinendegen, Strahlentherapie, 143, 468 (1972).

43. J. Pijck, J. Gillis, and J. Hoste, Int. J. Appl. Radiat. Isotopes, 10, 149 (1961).

44. J. Pijck and J. Hoste, "L'Analyse par Radioactivation et ses Applications aux Sciences Biologiques," Presses Universitaires de France, Paris, 1964, p. 361.

45. L.M. Paixao and J.H. Yoe, Clin. Chim. Acta, 4, 507 (1959).

46. R. Monacelli, H. Tanaka, and J.H. Yoe, Clin. Chim. Acta., 1, 577 (1956).

47. D. Comar, C. LePoec, M. Joly, and C. Kellershohn, Bull. Soc. Chim. Fr., 1, 56 (1962).

48. R.P. Spencer, T.G. Mitchell, and E.R. King, Amer. J. Roentgenol., 79, 1053 (1958).

49. S.M. Prigozhina, A.A. Kist, and E.M. Lobanov, "Aktiv. Anal. Biol. Ob'ektov 1967," p 92; Chem. Abstr., 70, 45594h (1969).

50. S.K. Teshabaev and E.N. Mavaeva, Tr. Samarkand. Gos. Univ., 193, 142 (1972), Chem. Abstr. 78, 107847v (1973).

51. K. Kasperek, H. Schica, A. Hoeck, V. Siller, and L.E. Feinendegen, Strahlentherapie, 145, 229 (1973).

52. R.E. Fredericks, K.R. Tanaka, and W.N. Valentine, J. Clin. Invest., 43, 304 (1964).

53. T.F. Budinger, J.R. Farwell, A.R. Smith, and H. Bichsel, Int. J. Appl. Radiat. Isotopes, 23, 49 (1972).

54. H.J.M. Bowen and P.A. Cawse, "The Determination of Inorganic Elements in Biological Tissue by Activation Anaysis," AERE-R-4309, Atomic Energy Research Establishment, Wantage, England, 1963.

55. R.A. Nadkarni, D.E. Fliedner, and W.D. Ehmann, Radiochim. Acta, 11, 97 (1969).

56. D. Brune, K. Samsahl, and P.O. Wester, Atompraxis, 9, 368 (1963); Chem. Abstr. 60, 7134c (1964).

57. D. Behne, J. Radioanal. Chem., 3, 17 (1969); Chem. Abstr. 71, 78061f (1969).

58. T. Asai, Y. Iwai, R. Miki, Y. Kondo, Y. Sato, and T. Azuma, Ann. Rep. Radiat. Center Osaka Prefect., 8, 120 (1967); Chem. Abstr. 69, 84012r (1968).

59. W.A. Haller, R. Filby, and L.A. Rancitelli, Nucl. Appl., 6, 365 (1969); Chem. Abstr. 71, 10161h (1969).

60. E.L. Kanabrocki, J. Nucl. Med., 6, 780 (1965).

61. G.C. Cotzias, S.T. Miller, and J. Edwards, J. Lab. Clin. Med., 67, 836 (1966).

62. H.J.M. Bowen, J. Nucl. Energy, 3, 18 (1956).

63. M. Simkova and M. Krivanek, J. Radioanal. Chem., 2, 229 (1969); Chem. Abstr. 70, 112270q (1969).

64. S. Meloni, A. Brandone, and V. Maxia, Int. J. Appl. Radiat. Isotopes, 20, 757 (1969).

65. H.J.M. Bowen, Analyst (London), 89, 658 (1964).

66. P.F. Johnson, P. Tothill and G.W.K. Donaldson, Intern. J. Appl. Radiat. Isotopes, 20, 103 (1969).

67. J. Savory, P. Mushak, F.W. Sunderman, Jr., R. Estes and N.O. Roszel, Anal. Chem., 42, 294 (1970).

68. J. Savory, P. Mushak and F.W. Sunderman, Jr., J. Chrom. Sci., 7, 674 (1969).

69. L.C. Hansen, W.G. Scribner, T.W. Gilbert, and R.E. Sievers, Anal. Chem., 43, 349 (1971).

70. W.R. Wolf, M.L. Taylor, B.M. Hughes, T.O. Tierman, and R.E. Sievers, Anal. Chem., 44, 616 (1972).

71. F.J. Feldman, G.D. Christian, and W.C. Purdy, Amer. J. Clin. Pathol., 49, 826 (1968).

72. S. Hamada, K. Torizuka, K. Hamamoto, T. Mori, R. Morita, T. Nakagawa, and J. Konishi, Radioisotopes, 18, 354 (1969); Chem. Abstr., 72, 63120w (1970).

73. R. Grunewald, P.L. Ziemer, and J.E. Christian, Anal. Chem., 36, 1138 (1964).

74. A. Dimitriadou, P.C.R. Turner, and T.R. Fraser, Nature, 198, 576 (1963).

75. A. Dimitriadou, P.C.R. Turner, & T.R. Fraser, Nature, 197, 446 (1963).

76. R.H. Tomlinson and M.W. Billinghurst, Trans. Amer. Nucl. Soc., 12, 458 (1969).

77. J.F. Balcius, D.M. Linekin and G.L. Brownell, Trans. Amer. Nucl. Soc., 12, 458 (1969).

78. R. McG. Harden and C.H. Bastomsky, Clin. Chem., 17, 1020 (1971).

79. H.J.M. Bowen, Biochem. J., 73, 381 (1959).

80. Y.K. Plotnikov, Terapevt. Arkh., 35, 79 (1963); Chem. Abstr., 59, 12013b (1963).

81. J.L.A. Hunteler, W. van der Silk, and J.P. Persijin, Clin. Chim. Acta, 36, 485 (1972).

82. H.Y. Yee and A. Zin, Clin. Chem., 17, 950 (1971).

83. L.O. Plantin, "L'Analyse par Radioactivation et ses Applications aux Sciences Biologiques," Presses Universitaries de France, Paris, 1964, p 211.

84. H.R. Lukens, K. Heydorn, and T. Choy, Trans. Amer. Nucl. Soc., 8, 331 (1965).

85. T.E.F. Carr and A. Sutton, in "Nuclear Activation Techniques in the Life Sciences," Proceedings of a Symposium, Amsterdam, 1967, IAEA, Vienna, 1967, p 445.

86. C. Jansen, G.W. Leddicotte, and M. Navarrette, Radiology, 91, 813 (1968).

87. T.M. Terree and S.H. Cohn, J. Nucl. Med., 7, 848 (1966).

88. B.S. Carpenter and C.H. Cheek, Anal. Chem., 42, 121 (1970).

89. B.S. Carpenter, in "Proceedings of the 1968 International Conference on Modern Trends in Activation Analysis," National Bureau of Standards, Gaithersburg Md., 1968, p 942.

90. M. Okuda and H. Sasmoto, Rinsho Byori, 17, 380 (1969); Chem. Abstr., 71, 78954f (1969).

91. H.D. Schwartz, B.C. McConville, and E.F. Christopherson, Clin. Chim. Acta, 31, 97 (1971).

92. S.O. Hansen and L. Theodorsen, Clin. Chim. Acta, 31, 119 (1971).

93. G.K. Davis, Ann. N.Y. Acad. Sci., 199, 92 (1972).

94. J. Pybus, F.J. Feldman, and G.N. Bowers, Jr., Clin. Chem., 16, 998 (1970).

95. P.A. Cawse and M. Daglish, Analyst (London), 89, 266 (1964).

96. C.K. Kim and W.W. Meinke, in "Proceedings of the 1965 International Conference on Modern Trends in Activation Analysis," College Station Texas, 1965, p 316.

97. D. Brune, Acta Chem. Scand., 20, 1200 (1966).

98. C. Kellershohn, D. Comar, and C. LePoec, J. Lab. Clin. Med., 66, 168 (1965).

99. B. Sjostrand, Anal. Chem., 36, 814 (1964).

100. J.Y. Hwang, P.A. Ullucci, and C.J. Mokeler, Anal. Chem., 45, 795 (1973).

101. G.A. Rose and E.G. Willden, Analyst (London), 98, 243 (1973).

102. M.M. Joselow and J.D. Bogden, At. Absorption Newslett., 11, 127 (1972).

103. H.J.M. Bowen and P.A. Cawse, Analyst (London), 88, 721 (1963).

104. R.C. Dickson and R.H. Tomlinson, Int. J. Appl. Radiat. Isotopes, 18, 153 (1967).

105. R.H. Tomlinson and R.C. Dickson, in "Proceedings of the 1965 International Conference on Modern Trends in Activation Analysis," College Station, Texas, 1965, p 66.

106. N.V. Bagdavadze, L.V. Barbakadze, E.N. Ginturi, N.E. Kuchava, L.M. Mosulishvili and N.E. Kharabadze, Soobshch. Akad. Nauk Gruz. SSR, 39, 287 (1965); Chem. Abstr, 64, 5439b (1966).

107. E. Sh. Botzvadze, L.M. Mosulishvili, N.E. Kuchava, and E.N. Ghinturi, Phys. Med. Biol., 14, 19 (1969).

108. J.V. Dunckley, Clin. Chem., 17, 992 (1971).

109. A. Abu-Samra and G.W. Leddicotte, in "Proceedings of the 1968 International Conference on Modern Trends in Activation Analysis," National Bureau of Standards, Gaithersburg, Md., 1968, p 134.

110. J. Pybus and G.N. Bowers, Jr., Clin. Chem., 16, 139 (1970).

111. H.J.M. Bowen, Biochem. J., 77, 79 (1960).

112. M.L. Taylor and E.L. Arnold, Anal. Chem., 43, 1328 (1971).

113. M.L. Taylor, E.L. Arnold, and R.E. Sievers, Anal. Lett., 1, 735 (1968).

114. J.F. Goodwin, Clin. Chem., 17, 544 (1971).

115. P. Venkateswarlu, L. Singer, and W.D. Armstrong, Anal. Biochem., 42, 350 (1971).

116. G.W. Leddicotte in "Methods of Biochemical Analysis," D. Glick, Ed., Interscience, New York, N.Y., 1971.

117. H.A. Schroeder and A.P. Nason, Clin. Chem., 17, 461 (1971).

118. H.J.M. Bowen, "Elementary Composition of Mammalian Blood," AERE-R-4196, Atomic Energy Research Establishment, Wantage, England, 1963; Chem. Abstr., 59, 6784c (1963).

119. R.A. Levine, D.H.P. Streeten, and R.J. Doisy, Metab., Clin. Exp., 17, 114 (1968).

120. W.B. Herring, B.S. Leavell, L.M. Paixao, and J.H. Yoe, Amer. J. Clin. Nutr., 8, 846 (1960).

121. P.O. Wester, "Trace Elements in Human Myocardial Infarction Determined by Neutron Activation Analysis," AE-188, Aktiebolaget Atomenergi, Stockholm, Sweden, 1965.

122. P. Correa and J.P. Strong, Ann. N.Y. Acad. Sci., 199, 217 (1972).

123. P.O. Wester, Acta Med. Scand., 178, 765 (1965).

124. H.A. Schroeder, J. Amer. Med. Ass., 172, 1902 (1960).

125. G. Biorck, H. Bostrom, and A. Widstrom, Acta Med. Scand,. 178, 239 (1965).

126. H.M. Perry, Jr., Ann. N.Y. Acad. Sci., 199, 202 (1972).

127. G.L. Curran, J. Biol. Chem., 210, 765 (1954).

128. J.W. Berg and F. Burbank, Ann. N.Y. Acad. Sci., 199, 249 (1972).

129. W.J. Pories, E.G. Mansour, and W.H. Strain, Ann. N.Y. Acad. Sci., 199, 265 (1972).

130. L. de Galan, "Analytical Spectroscopy," Adam Hilger, Ltd., London, 1971, p 127.

131. H.L. Kahn in "Trace Inorganics in Water," Advances in Chemistry Series, No. 73, R.F. Gould, Ed., American Chemical Society, Washington, D.C., 1968, p 183.

132. D.C. Manning and F. Fernadez, At. Absorption Newslett., 9, 65 (1970).

133. J.D. Winefordner and R.C. Elser, Anal. Chem., 43 (4), 24A (1971).

134. B.V. L'vov, Spectrochim. Acta, 24B, 53 (1969).

135. V.A. Fassel and D.W. Golightly, Anal. Chem., 39, 466 (1967).

136. E.E. Pickett and S.R. Koirtyohann, Spectrochim. Acta, 23B, 235 (1968).

137. E.E. Pickett and S.R. Koirtyohann, Spectrochim. Acta, 24B, 325 (1969).

138. S. Greenfield and P.B. Smith, Anal. Chim. Acta, 59, 341 (1972).

139. J.D. Winefordner, V. Svoboda, and L.J. Cline, Crit. Rev. Anal. Chem., 1, 233 (1970).

140. A. J. Mitteldorf, in "Trace Analysis, Physical Methods," G.H. Morrison, Ed., Interscience, New York, N.Y., 1965, p 193.

141. J.D. Winefordner, S.G. Schulman, and T.C. O'Haver, "Luminescence Spectrometry in Analytical Chemistry," Interscience, New York, N.Y., 1972.

142. F.S. Goulding and J.M. Jaklevic, "Trace Element Analysis by X-Ray Fluorescence," URCL-20625, Lawrence Radiation Laboratory, 1971.

143. R.D. Giauque, F.S. Goulding, J.M. Jaklevic, and R.H. Pehl, Anal. Chem., 45, 671 (1973).

144. F.C. Young, M.L. Roush, and P.G. Berman, Int. J. Appl. Radiat. Isotopes, 24, 153 (1973).

145. I. Adler and H.J. Rose, Jr., in "Trace Analysis, Physical Methods," G.H. Morrison, Ed., Interscience, New York, N.Y., 1965, p 271.

146. L.S. Birks, Anal. Chem., 44(5), 557R (1972).

147. R.H. Hammerle, R.H. Marsh, K. Rengan, R.D. Giauque, and J.M. Jaklevic, Anal. Chem., 45, 1939 (1973).

148. D.E. Leyden, R.E. Channell and C.W. Blount, Anal. Chem., 44, 607 (1972).

149. D.E. Porter and R. Woldseth, Anal. Chem., 45, 604, (1973).

150. J. Reboz, in "Trace Analysis, Physical Methods," G.H. Morrison, Ed., Interscience, New York, N.Y., 1965, p 435.

151. L. de Galan, "Analytical Spectrometry," Adam Hilger, Ltd., London, 1971, p 241.

152. N.M. Frew, J.J. Leary, and T.L. Isenhour, Anal. Chem., 44, 665 (1972).

153. P.J. Paulsen, R. Alvarez, and D.E. Kellerher, Spectrochim. Acta, 24B, 535 (1969).

154. L.J. Moore and L.A. Machlan, Anal. Chem., 44, 2291 (1972).

155. N. Gochman and D.S. Young, Anal. Chem., 45, 11R (1973).

156. H.B. Mark, Jr., J. Pharm. Belg., 25, 367 (1967).

157. J.D. Czaban and G.A. Rechnitz, Anal. Chem., 45, 471 (1973).

158. G.J. Patriarche, Anal. Lett., 5, 45 (1972).

159. F.M. Wachi, Diss. Abstr., 20, 53 (1959).

160. H. Freiser, Anal. Chem., 31, 1440 (1959).

161. A.A. Duswalt, Jr., Diss. Abstr., 20, 52 (1959).

162. N.D. Sokolov, M.A. Baydarovtseva, and N.A. Vakin, Izv. Akad. Nauk SSSR, Ser. Khim., No. 6, 1396 (1968).

163. R.W. Moshier and R.E. Sievers, "Gas Chromatography of Metal Chelates," Pergamon Press, New York, N.Y., 1965, p 42.

164. S.T. Sie, J.P.A. Bleumer, and G.W.A. Rijnders, Separ. Sci., 1, 41 (1966).

165. S.T. Sie, J.P.A. Bleumer, and G.W.A. Rijnders, Separ. Sci., 2, 645 (1967).

166. S.T. Sie, J.P.A. Bleumer, and G.W.A. Rijnders, Separ. Sci., 3, 165 (1968).

167. R.W. Moshier and R.E. Sievers, "Gas Chromatography of Metal Chelates," Pergamon Press, New York, N.Y., 1965, p 2.

168. L.M. Brown and K.S. Mazdiyasni, Anal. Chem., 41, 1243 (1969).

169. R.W. Moshier and R.E. Sievers, "Gas Chromatography of Metal Chelates," Pergamon Press, New York, N.Y., 1965, p 40.

170. W.C. Butts, Diss. Abstr., 29, 506B (1968).

171. T. Shigematou, M. Matsui, and K. Utsunomiya, Bull. Chem. Soc. Jap., 41, 763 (1968).

172. R.E. Sievers, K.J. Eisentraut, and C.S. Springer, in "Lanthanide/Actinide Chemistry," Advances in Chemistry Series, No. 71, R.F. Gould, Ed., American Chemical Society, Washington, D.C., 1967, p 141.

173. M. Tanaka, T. Shono, and K. Shinra, Anal. Chim. Acta, 43, 157 (1968).

174. C.S. Springer, Jr., D.W. Meek, and R.E. Sievers, Inorg. Chem., 6, 1105 (1967).

175. W.G. Scribner and R.E. Sievers, Personal Communication.

176. C. Genty, C. Houin, and R. Schott, 7th International Symposium on Gas Chromatography and its Exploitation, Copenhagen, Denmark, June 1968.

177. W.G. Scribner, W.J. Treat, J.D. Weis, and R.W. Moshier, Anal. Chem., 37, 1136 (1965).

178. W.G. Scribner, M.J. Borchers, and W.J. Treat, Anal. Chem., 38, 1179 (1966).

179. W.D. Ross and R.E. Sievers, Anal. Chem., 41, 1109 (1969).

180. R.E. Sievers, G. Wheeler, Jr., and W.D. Ross, Proceedings, 3rd International Symposium on Advances in Gas Chromatography, Houston, Texas, 1965.

181. W.D. Ross and R.E. Sievers, Talanta, 15, 87 (1968).

182. R.W. Moshier and J.E. Schwarberg, Talanta, 13, 445 (1966).

183. G.P. Morie and T.R. Sweet, Anal. Chim. Acta, 34, 314 (1966).

184. G.P. Morie and T.R. Sweet, Anal. Chem., 37, 1553 (1965).

185. J.E. Schwarberg, R.W. Moshier, and J.H. Walsh, Talanta, 11, 1213 (1964).

186. R.D. Hill and H. Gesser, J. Gas Chromatogr., 1, 11 (1963).

187. R.E. Sievers, B.W. Ponder, M.L. Morris, and R.W. Moshier, Inorg. Chem., 2, 693 (1963).

188. W.D. Ross and G. Wheeler, Jr., Anal. Chem., 36, 266 (1964).

189. W.D. Ross, Anal. Chem., 35, 1596 (1963).

190. R.W. Moshier and R.E. Sievers, "Gas Chromatography of Metal Chelates," Pergamon Press, New York, N.Y., 1965, p 41.

191. R.W. Moshier and R.E. Sievers, "Gas Chromatography of Metal Chelates," Pergamon Press, New York, N.Y., 1965, p 53.

192. R.E. Sievers, J.W. Connolly, and W.D. Ross, J. Gas Chromatogr., 5, 241 (1967).

193. W.D. Ross, W.G. Scribner, and R.E. Sievers, Personal Communication.

194. D.K. Albert, Anal. Chem., 36, 2034 (1964).

195. R.A. Nadkarni and G.H. Morrison, Anal. Chem., 45, 1957 (1973).

196. T. Asai, Y. Iwai, R. Miki, Y. Kondo, Y. Sato and T. Azuma, Ann. Rep. Radiat. Center Osaka Prefect., 6, 111 (1965); Chem. Abstr. 66, 62578r (1967).

197. D.C. Borg, R.E. Segel, P. Kienle and L. Campbell, Int. J. Appl. Radiat. Isotopes, 11, 10 (1961).

198. R.D. Cooper and G.L. Brownell, Nucl. Instrum. Methods, 51, 72 (1967).

199. H.P. Yule, Anal. Chem., 38, 818 (1966).

200. W.W. Meinke, "L'Analyse par Radioactivation et ses Applications aux Sciences Biologiques," Presses Universitaires de France, Paris, 1964, p 145.

201. T.T. Gorsuch, Analyst (London), 84, 135 (1959).

202. D.S. Robertson, Nature, 210, 1357 (1966).

203. H.J.M. Bowen, Analyst (London), 92, 118 (1967).

204. K. Heydorn and E. Damsgaard, Talanta, 20, 1 (1973).

205. M. Rakovic, Chem. Zvesti, 23, 147 (1969); Chem. Abstr. 71, 687v (1969).

206. M. Rakovic, I. Langerova and H. Talpova, Atompraxis, 12, 344 (1966); Chem. Abstr., 65, 18983e (1966).

207. M. Rakovic and Z. Prochazkova, Nucl. Med., 5, 436 (1966).

208. M. Rakovic, Chem. Zvesti, 20, 293 (1966); Chem. Abstr., 65, 2987d (1966).

209. H.J.M. Bowen and P.A. Cawse, Analyst (London), 86, 506 (1961).

210. L. Kosta and A.R. Byrne, Talanta, 16, 1297 (1969).

211. W. Gebaur, Radiochim. Acta, 4, 191 (1965).

212. W.J. Ross, Anal. Chem., 36, 1114 (1964).

213. K. Samsahl, Anal. Chem., 39, 1480 (1967).

214. K. Samsahl, "A Fast Radiochemical Method for the Determination of Some Essential Trace Elements in Biology and Medicine," AE-168, Aktiebolaget Atomenergi, Stockholm, Sweden, 1964.

215. F.J. Berlandi, Diss. Abstr., 27, 2264B (1966).

216. J.I. Kim and H. Stark, Radiochim. Acta, 13, 213 (1970).

217. D. Behne, Radiochem. Radioanal. Lett., 6, 39 (1971).

218. D. Behne, Kerntechnik, 12, 112 (1970).

219. F. Grass and R. Kittl, Mikrorchim. Acta, 371 (1971).

220. K.G. Kjellin, in "Nuclear Activation Techniques in the Life Sciences," Proceedings of a Symposium, Amsterdam, 1967, IAEA, Vienna, 1967, p 517.

221. J. Op de Beeck, Anal. Chim. Acta, 40, 221 (1968).

222. E.L. Kanabrocki, L.F. Case, T. Fields, L. Graham, E.B. Miller, Y.T. Oester and E. Kaplan, J. Nucl. Med., 6, 489 (1965).

223. E.L. Kanabrocki, L.F. Case, T. Fields, L. Graham, E.B. Miller, Y.T. Oester, and E. Kaplan, J. Nucl. Med., 6, 780 (1965).

224. E.L. Kanabrocki, L.F. Case, E.B. Miller, E. Kaplan and Y.T. Oester, J. Nucl. Med., 5, 643 (1964).

225. G.J. Olivares, Acta Cient. Venez., 18, 9 (1967).

226. T. Freeman and J. Smith, Biochem. J., 118, 869 (1970).

227. C. Veselsky, M. Nedbalek, and O. Suschny, Int. J. Appl. Radiat. Isotopes, 21, 225 (1970).

228. K. Fritze, and R. Robertson, J. Radioanal. Chem., 1, 463 (1968).

229. T.K. Lin and S.J. Yeh, Nucl. Sci. Technol. (Tokyo), 3, 289 (1966).

230. J. Torko, Energ. Atomtech., 20, 200 (1967).

231. D. Brune, "Recoil Reactions in Neutron Activation Analysis. I. The Szilard-Chalmers Effect Applied in the Analysis of Biological Samples. II. Transfer of Activities from Container Materials to Sample," AE-172, Aktiebolaget Atomenergi, Stockholm, Sweden, 1965; Chem. Abstr., 63, 2113 (1965).

232. D. Brune, Anal. Chim. Acta, 34, 447 (1966).

233. D. Comar, and C. Le Poec, in "Proceedings of the 1964 Symposium on Radiochemical Methods of Analysis," Salzburg, Austria, 1965, p 15.

234. J. Shankar, K.S. Venkateswarlu and M. Lal, J. Inorg. Nucl. Chem., 28, 11 (1966).

235. K.A. Rao and N. Nath, Radiochim. Acta, 5, 162 (1966).

236. E. Lazzarini, J. Inorg. Nucl. Chem., 29, 7 (1967).

237. I. Gainar and A. Ponta, Rev. Roum. Chim., 13, 401 (1968).

238. J. Shankar, "Hot Atom Chemistry: Annealing of Recoil Damage and Solid State Isotopic Exchange in Cobalt Complexes," BARC-348, Bhabha Atomic Research Centre, Bombay, India, 1968.

239. J. Shankar, A. Nath and V.G. Thomas, J. Inorg. Nucl. Chem., 30, 1361 (1968).

240. G.H. Morrison and H. Freiser, "Solvent Extraction in Analytical Chemistry," J. Wiley and Sons, New York, N.Y., 1962.

241. J. Stary, "The Solvent Extraction of Metal Chelates," Pergamon Press, New York, N.Y., 1964.

242. F. Kukula, B. Mudrova and M. Krivanek, Talanta, 14, 233 (1967).

243. A. Alian and R. Parthasarathy, Anal. Chim. Acta, 35, 69 (1966).

244. K.J. Hahn, D.J. Tuma and J.L. Sullivan, Anal. Chem., 40, 974 (1968).

245. K.J. Hahn, D.J. Tuma and M.A. Quaife, Anal. Chem., 39, 1169 (1967).

246. H. Green, Talanta, 20, 139 (1973).

247. S. Ohno, Analyst (London), 96, 423 (1971).

248. H. Wesch, J. Zimmerer and J. Schmuamacher, Int. J. Appl. Radiat. Isotopes, 21, 431 (1970).

249. F. Girardi, in "Nuclear Activation Techniques in the Life Sciences," Proceedings of a Symposium, Amsterdam, 1967, IAEA, Vienna, 1967, p 117.

250. F. Girardi, R. Pietra and E. Sabbioni, in "Proceedings of the 1968 International Conference on Modern Trends in Activation Analysis," National Bureau of Standards, Gaithersburg Md., 1968, p 639.

251. F. Girardi, R. Pietra, and E. Sabbioni, J. Radioanal. Chem., 5, 141 (1970).

252. F. Girardi, R. Pietra and E. Sabbioni, "Radiochemical Separation by Retention on Ionic Precipitates. Adsorption Tests on 17 Materials," EUR-4287; Chem. Abstr. 72, 73549g (1970).

253. C. Bigliocca, F. Girardi, J. Pauly, E. Sabbioni, S. Meloni and A. Provasoli, Anal. Chem., 39, 1634 (1967).

254. F. Girardi and E. Sabbioni, J. Radioanal. Chem., 1, 169 (1968).

255. C.W. Tang and C.J. Maletskos, Science, 167, 52 (1970).

256. C.K. Kim and J. Silverman, Anal. Chem., 37, 1616 (1965).

257. C.K. Kim and J. Silverman, Trans. Amer. Nucl. Soc., 7, 332 (1964).

258. D. Brune, Acta Chem. Scand., 20, 1200 (1966).

259. D. Brune and K. Jirlow, Radiochim. Acta, 8, 161 (1967).

260. R.R. Ruch and R. DeVoe, Anal. Chem., 39, 1333 (1967).

261. J.R. DeVoe, C.K. Kim and W.W. Meinke, Talanta, 3, 298 (1960).

262. D.L. Massart, "Cation-Exchange Techniques in Radiochemistry," NAS-NS-3113 (Rev.), Nuclear Science Series of the National Academy of Sciences-National Research Council, Washington, D.C., 1971.

263. J. Inczedy, "Analytical Applications of Ion Exchangers," Pergamon Press, New York, N.Y., 1966.

264. G. Schmuckler, Talanta, 12, 281 (1965).

265. J.P. Riley and D. Taylor, Anal. Chim. Acta, 40, 479 (1968).

266. C.W. Blount, D.E. Leyden, T.L. Thomas and S.M. Guill, Anal. Chem., 45, 1045 (1973).

267. K. Fritze, N. Aspin, and T.H. Holmes, Radiochim. Acta., 3, 204 (1964).

268. G. Aubouin and J. Laverlochere, J. Radioanal. Chem., 1, 123 (1968).

269. R. Griessbach, Angew. Chem., 67, 606 (1955).

270. R. Dybczynski, J. Chromatogr., 31, 155 (1967).

271. F. Tera, R.R. Ruch and G.H. Morrison, Anal. Chem., 37, 358 (1965).

272. F. Tera and G.H. Morrison, Anal. Chem., 38, 959 (1966).

273. J.C. Ricq, J. Radioanal. Chem., 1, 443 (1968).

274. V.V. Moiseev, R.A. Kuznetsov and A.I. Kalinin, in "Proceedings of the 1965 International Conference on Modern Trends in Activation Analysis," College Station, Texas, 1965, p 164.

275. D. Comar and C. Le Poec, in "Proceedings of the 1965 International Conference on Modern Trends in Activation Analysis," College Station, Texas, 1965, p 351.

276. K. Samsahl and D. Brune, Int. J. Appl. Radiat. Isotopes, 16, 273 (1965).

277. K. Samsahl, P.O. Wester and O. Landstrom, Anal. Chem., 40, 181 (1968).

278. K. Samsahl, "High-Speed, Automatic Radiochemical Separations for Analysis in the Biological and Medical Research Laboratory," AE-389, Aktiebolaget Atomenergi, Stockholm, Sweden, 1970; Chem. Abstr., 73, 95361n (1970).

279. K. Samsahl, Nukleonic, 8, 252 (1966).

280. G.H. Morrison, J.T. Gerard, A.T. Kashuba, E.V. Gangadharam, A.M. Rothenberg, N.M. Potter and G.B. Miller, Science, 167, 505 (1970).

281. G.H. Morrison, J.T. Gerard, A. Travesti, R.L. Currie, S.F. Peterson and N.M. Potter, Anal. Chem., 41, 1633 (1969).

282. F. Girardi, Cron. Chim., No. 27, 14 (1970); Chem. Abstr., 73, 136447r (1970).

283. P. Vanderkooi, B. Heagan, and J.T. Lowman, Proc. Soc. Exp. Biol. Med., 113, 772 (1963).

284. J. Hadzistelios and C. Papadopoulou, Talanta, 16, 337 (1969).

285. P. Van den Winkel, A. Speecke and J.J. Hoste, in "Nuclear Activation Techniques in the Life Sciences," Proceedings of a Symposium, Amsterdam, 1967, IAEA, Vienna, 1967, p 569.

286. N. Spronk, in "Nuclear Activation Techniques in the Life Sciences," Proceedings of a Symposium, Amsterdam, 1967, IAEA, Vienna, 1967, p 335.

287. M. Heurtebise, J. Radioanal. Chem., 7, 227 (1971).

288. M.H. Feldman, J. McNamara, R.C. Reba and W. Webster, J. Nucl. Med., 8, 123 (1967).

289. M. Heurtebise and W.J. Ross, Anal. Chem., 43, 1438 (1971).

290. R.E. Jervis and K.Y. Wong, in "Nuclear Activation Techniques in the Life Sciences," Proceedings of a Symposium, Amsterdam, 1967, IAEA, Vienna, 1967 p 137.

291. U. Eisner, J.M. Rottschafer, F.J. Berlandi and H.B. Mark, Jr., Anal. Chem., 38, 1466 (1967).

292. A.Z. Budzynski and J.Z. Beer, Anal. Chim. Acta, 46, 281 (1969).

293. T. Nascutiu, Rev. Roum. Chim., 9, 283 (1964).

294. T. Nascutiu, Acad. Repub. Pop. Rom., Stud. Cercet, Chim., 10, 275 (1962; Chem. Abstr. 58, 2841g (1963).

295. W. Bock-Werthmann, Anal. Chim. Acta, 28, 519 (1963).

296. M. Qureshi and S.D. Sharma, Anal. Chem., 45, 1283 (1973).

297. J.M. Steim and A.A. Benson, Anal. Biochem, 9, 21 (1964).

298. J.M. Steim, Diss. Abstr., 23, 3622 (1963).

299. A.A. Benson, W.W. Miller, and J.M. Steim, Nippon Isotope Kaigi Hobun-shu, 119 (1963).

300. R. Blomstrand and F. Nakayama, J. Neurochem., 8, 230 (1961).

301. J.B. Smathers, D. Duffy and S. Lakshamanan, Anal. Chim. Acta, 46, 9 (1969).

302. H. Staerk and D. Knorr, Atomkernenergie, 6, 408 (1961); Chem. Abstr., 56, 3765b (1962).

303. J.B. Smathers, D. Duffy and S. Lakshmanan, Anal. Chim. Acta, 39, 529 (1967).

304. W.T. Burnett, Jr., and M.D. Cohan, Trans. Amer. Nucl. Soc., 7, 333 (1964).

305. L.S. Bark, G. Duncan and R.J.T. Graham, Analyst (London), 92, 347 (1967).

306. L.S. Bark, G. Duncan, and R.J.T. Graham, Analyst (London), 92, 31 (1967).

307. S.P. Cram, Advan. Chromatogr., 9, 243 (1970).

308. S.P. Cram and J.L. Brownlee, Jr., J. Gas Chromatogr., 5, 353 (1967).

309. S.P. Cram and J.L. Brownlee, Jr., J. Gas Chromatogr., 6, 305 (1968).

310. S.P. Cram and J.L. Brownlee, Jr., J. Gas. Chromatogr., 6, 313 (1968).

311. D.B. Cottrell, Ph.D. Thesis, The University of Florida, Gainesville, Florida, 1973.

312. E.W. Berg and J.T. Truemper, J. Phys. Chem., 64, 487 (1960).

313. M. Tanaka, T. Shono, and K. Shinra, Nippon Kagaku Zasshi, 89, 669 (1968).

314. R.W. Moshier and R.E. Sievers, "Gas Chromatography of Metal Chelates," Pergamon Press, New York, N.Y. 1965, p. 4.

315. C.E. Crouthamel, "Applied Gamma-Ray Spectrometry," 2nd ed, completely revised and enlarged by F. Adams and R. Dams, Pergamon Press, Ltd., Elmsford, N.Y. 1970.

316. J.P. Collman, in "Inorganic Synthesis," Vol 7, McGraw-Hill Book Company, New York, N.Y., 1963, p 134.

317. R.W. Moshier and R.E. Sievers, "Gas Chromatography of Metal Chelates," Pergamon Press, New York, N.Y., 1965, p 118.

BIOGRAPHICAL SKETCH

Frederick Turner Varcoe, Jr., was born April 27, 1944, at Chattanooga, Tennessee. In 1953 he and his parents, an actuary and a mathematics instructor, moved to Indianapolis, Indiana. He returned to Chattanooga to attend The McCallie School, graduating in May, 1962. He and his family then moved to Jackson, Mississippi, where he received the degree of Bachelor of Science cum laude from Millsaps College in June, 1966. He entered the University of Florida in September, 1966, and since that time he has been working toward the degree of Doctor of Philosophy.

While in Gainesville he was active in the Gator Sail Club, the Gator Ski Club, and worked in a drug abuse rehabilitation program at the Drug Project. He is a member of the American Chemical Society and Alpha Chi Sigma.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Roger G. Bates, Chairman
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James D. Winefordner
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert J. Hanrahan
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John H. James
Assoc. Professor of Management

This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1974

_____

Dean, Graduate School